

10-July-2001

APPENDIX A

ROBUST SUMMARY FOR ADIPONITRILE

The studies listed below were selected to represent the best available study design and execution for these HPV toxicity endpoints. Other data of equal or lesser quality are not summarized, but are listed as additional references in this document.

1.0 Substance Information

CAS Number: 111-69-3

Chemical Name: Hexanedinitrile

Structural Formula: $\text{N}\equiv\text{C}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{C}\equiv\text{N}$

Other Names: 1,4-Dicyanobutane
Adipic acid dinitrile
Adipic acid nitrile
Adipic dinitrile
Adipodinitrile
Adiponitrile
Adipyldinitrile
ADN
Dinitrilo-1,6 hexane
Hexanedioic acid, dinitrile
Nitrile adipique
Tetramethylene cyanide
Tetramethylene dicyanide

Exposure Limits: 2 ppm, (8- and 12-hour TWA), skin: DuPont Acceptable Exposure Limit (AEL)

2 ppm (8.8 mg/m³), skin: ACGIH Threshold Limit Value (TLV)

10 mg/m³ STEL January, 1993: Occupational Exposure Limit (OEL) - Russia

4 ppm (18 mg/m³) (10-hour TWA): NIOSH REL

2.0 Physical /Chemical Properties

2.1 Melting Point

Value: 1°C

Decomposition: No Data

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Sublimation: No Data
Pressure: No Data
Method: No Data
GLP: Unknown
Reference: Lide, D. R. (1990-1991). CRC Handbook of Chemistry and Physics, 71st ed., p. 3-29, CRC Press, Boca Raton, FL (HSDB/627).
Reliability: Not assignable because limited study information was available.

Additional References for Melting Point:

Henry (1902). Rec. Trav. Chim. Pays-Bas, 21:2 (cited in IUCLID (2000). IUCLID Data Sheet "adiponitrile" (February 18)).

Anon. (1901). C. Zentralblatt, II, p. 807 (cited in IUCLID (2000). IUCLID Data Sheet "adiponitrile" (February 18)).

Verschueren, K. (1977). Handbook of Environmental Data on Organic Chemicals, Van Nostrand Reinhold Co., New York.

Smiley, R. A. (1981). Kirk-Othmer Encycl. Chem. Tech., 3rd ed., Vol. 15, p. 897-901, John Wiley and Sons, New York (cited in US EPA, Health and Environmental Effects Document for Adiponitrile, ECAO-CIN-G024).

2.2 Boiling Point

Value: 295°C
Decomposition: No Data
Pressure: 760 mm Hg
Method: No Data
GLP: Unknown
Reference: Lide, D. R. (1990-1991). CRC Handbook of Chemistry and Physics, 71st ed., p. 3-29, CRC Press, Boca Raton, FL (HSDB/627).
Reliability: Not assignable because limited study information was available.

Additional References for Boiling Point:

Henry (1902). Rec. Trav. Chim. Pays-Bas, 21:2 (cited in IUCLID (2000). IUCLID Data Sheet "adiponitrile" (February 18)).

Anon. (1901). C. Zentralblatt, II, p. 807 (cited in IUCLID (2000). IUCLID Data Sheet "adiponitrile" (February 18)).

Kulikowa et al. (1959). Zh. Prikl. Khim (Lenigrad), 32:227-229, english translation, p. 234 (cited in IUCLID (2000). IUCLID Data Sheet “adiponitrile” (February 18)).

Thorpe (1909). J. Chemi. Soc., 95:1902 (cited in IUCLID (2000). IUCLID Data Sheet “adiponitrile” (February 18)).

Union Chimique – Chem Bedrijren, ZA 6707122 (1968). (CA70:57171f).

Verschueren, K. (1977). Handbook of Environmental Data on Organic Chemicals, Van Nostrand Reinhold Co., New York.

Smiley, R. A. (1981). Kirk-Othmer Encycl. Chem. Tech., 3rd ed., Vol. 15, p. 897-901, John Wiley and Sons, New York (cited in US EPA, Health and Environmental Effects Document for Adiponitrile, ECAO-CIN-G024).

2.3 Density

Value:	0.9676 g/mL
Temperature:	20°C
Method:	No Data
GLP:	Unknown
Results:	No additional data.
Reference:	Lide, D. R. (1990-1991). <u>CRC Handbook of Chemistry and Physics</u> , 71 st ed., p. 3-29, CRC Press, Boca Raton, FL (HSDB/627).
Reliability:	Not assignable because limited study information was available.

Additional References for Density:

Clayton, G. D. and F. E. Clayton (1982). Patty's Industrial Hygiene and Toxicology, 3rd ed., Vol. 2C, John Wiley and Sons, Inc., New York (cited in IUCLID (2000). IUCLID Data Sheet “adiponitrile” (February 18)).

Sax, N. I. and R. J. Lewis (1988). Dangerous Properties of Industrial Materials, 7th ed., Vol. II, Van Nostrand Reinhold, New York (cited in IUCLID (2000). IUCLID Data Sheet “adiponitrile” (February 18)).

Verschueren, K. (1977). Handbook of Environmental Data on Organic Chemicals, Van Nostrand Reinhold, New York (cited in IUCLID (2000). IUCLID Data Sheet “adiponitrile” (February 18)).

Weast, R. C. and J. G. Grasselli (1989). Handbook of Data on Organic Chemicals, 2nd ed., Vol. IV (cited in IUCLID (2000). IUCLID Data Sheet “adiponitrile” (February 18)).

Rhone-Poulenc (n.d.). Safety Data Sheet (cited in IUCLID (2000). IUCLID Data Sheet “adiponitrile” (February 18)).

Smiley, R. A. (1981). Kirk-Othmer Encycl. Chem. Tech., 3rd ed., Vol. 15, p. 897-901, John Wiley and Sons, New York (cited in US EPA, Health and Environmental Effects Document for Adiponitrile, ECAO-CIN-G024).

2.4 Vapor Pressure

Value: 6.8×10^{-4} mm Hg
Temperature: 25°C
Decomposition: No Data
Method: No Data
GLP: Unknown
Reference: Daubert, T. E. and R. P. Danner (1989). Physical and Thermodynamic Properties of Pure Chemicals Data Compilation, Taylor and Francis, Washington, DC (HSDB/627).
Reliability: Not assignable because limited study information was available.

Additional References for Vapor Pressure:

Rhone-Poulenc (n.d.). Safety Data Sheet (cited in IUCLID (2000). IUCLID Data Sheet “adiponitrile” (February 18)).

Lide, D. R. (1990-1991). CRC Handbook of Chemistry and Physics, 71st ed., p. 3-29, CRC Press, Boca Raton, FL (cited in IUCLID (2000). IUCLID Data Sheet “adiponitrile” (February 18)).

Verschueren, K. (1977). Handbook of Environmental Data on Organic Chemicals, Van Nostrand Reinhold Co., New York.

Neely, W. B. and G. E. Blau (1985). Environmental Exposure from Chemicals, Vol. 1, p. 31, CRC Press, Inc., Boca Raton, FL (cited in US EPA, Health and Environmental Effects Document for Adiponitrile, ECAO-CIN-G024).

2.5 Partition Coefficient (log K_{ow})

Value: -0.32
Temperature: 25°C
Method: OECD Guideline 107 “Partition Coefficient (n-octanol/water), Flask-shaking Method”
GLP: Unknown
Reference: BASF AG (1988). Analytisches labor, unveroeffentlichte

untersuchungen (J.Nr 101807 vom December 8) (cited in IUCLID (2000). IUCLID Data Sheet “adiponitrile” (February 18)).

Reliability: Not assignable because limited study information was available.

Additional References for Partition Coefficient (log Kow):

Tanii, H. and K. Hashimoto (1985). Arch. Toxicol., 57(2):88-93 (cited in IUCLID (2000). IUCLID Data Sheet “adiponitrile” (February 18)).

Tanii, H. and K. Hashimoto (1932). Toxicol. Lett., 11:125-129 (cited in IUCLID (2000). IUCLID Data Sheet “adiponitrile” (February 18)).

Leo, A. J. (1978). Report on the Calculation of Octanol/Water Log P Values for Structures in EPA Files (ISHOW/305663).

U.S. EPA (1987). Graphical Exposure Modeling System (GEMS). CLOGP computer program, Office of Toxic Substances, U.S. EPA, Washington, DC (cited in US EPA, Health and Environmental Effects Document for Adiponitrile, ECAO-CIN-G024).

2.6 Water Solubility

Value: 8 wt% (80 g/L)

Temperature: 20°C

pH/pKa: No Data

Method: No Data

GLP: Unknown

Reference: Smiley, R. A. (1981). Kirk-Othmer Encycl. Chem. Tech., 3rd ed., Vol. 15, p. 897-901, John Wiley and Sons, New York (HSDB/627).

Reliability: Not assignable because limited study information was available.

Additional References for Water Solubility:

Rhone-Poulec (n.d.). Safety Data Sheet (cited in IUCLID (2000). IUCLID Data Sheet “adiponitrile” (February 18)).

U.S. Coast Guard (1974). Department of Transportation, CHRIS: Part 2 – Chemical Hazards Data, CG-446-2 (ISHOW/305662).

2.7 Flash Point

Value: 159°C
Method: Closed Cup; NFT 60-103 French Standard – Flash Point, in closed cup, of lubricants and combustible oils
GLP: No
Reference: Butachimie Courbevoie Cedex (n.d.). (cited in IUCLID (2000). IUCLID Data Sheet “adiponitrile” (February 18)).
Reliability: Not assignable because limited study information was available.

Additional References for Flash Point:

NFPA (National Fire Protection Association) (1991). National Fire Protection Guide. Fire Protection Guide on Hazardous Materials, 10th ed., p. 49-20, NFPA, Quincy, MA.

Smiley, R. A. (1981). Kirk-Othmer Encycl. Chem. Tech., 3rd ed., Vol. 15, p. 897-901, John Wiley and Sons, New York (cited in US EPA, Health and Environmental Effects Document for Adiponitrile, ECAO-CIN-G024).

2.8 Flammability

Results: 1.7 – 5.0% (in air); Autoignition temperature = 550°C
Method: No
GLP: Unknown
Reference: Rhone-Poulenc (n.d.). Safety Data Sheet (cited in IUCLID (2000). IUCLID Data Sheet “adiponitrile” (February 18)).
Reliability: Not assignable because limited study information was available.

Additional References for Flammability:

NFPA (National Fire Protection Association) (1991). National Fire Protection Guide. Fire Protection Guide on Hazardous Materials, 10th ed., p. 49-20, NFPA, Quincy, MA (HSDB/627).

U.S. Coast Guard (1985-1985). Department of Transportation, CHRIS – Hazardous Chemical Data, Volume II, U.S. Government Printing Office, Washington, DC (HSDB/627).

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3.0 Environmental Fate

3.1 Photodegradation

Concentration:	Not Applicable
Temperature:	Not Applicable
Direct	Not Applicable
Photolysis:	
Indirect	Not Applicable
Photolysis:	
Breakdown	Not Applicable
Products:	
Method:	If released to the atmosphere, adiponitrile is expected to exist almost entirely in the vapor phase. The rate constant for the reaction of adiponitrile vapor with photochemically generated hydroxyl radicals in the atmosphere has been estimated to be 1.4×10^{-12} cm ³ /molecule-sec at 25°C (Atkinson, 1987; SRC, n.d.). This value corresponds to a reaction half-life of 11.6 days assuming an ambient hydroxyl radical concentration of 5×10^5 molecules/cm ³ under typical atmospheric conditions (Atkinson, 1987; SRC, n.d.). A water solubility of 8×10^4 mg/L at 20°C (Smiley, 1981) also suggests that some loss by wet deposition may also occur (SRC, n.d.).
GLP:	Not Applicable
Reference:	Atkinson, R. (1987). <u>Intern. J. Chem. Kinet.</u> , 19:799-828 (HSDB/627). Smiley, R. A. (1981). <u>Kirk-Othmer Encycl. Chem. Tech.</u> , 3 rd ed., Vol. 15, p. 897-901, John Wiley and Sons, New York (HSDB/627). SRC (Syracuse Research Corporation) (n.d.). (HSDB/627).
Reliability:	Estimated value based on accepted model.

Additional Reference for Photodegradation:

Data from this additional source were not summarized because insufficient information was available.

Butachimie (1992). Report on the photodegradation of Adiponitrile (cited in IUCLID (2000). IUCLID Data Sheet "adiponitrile" (February 18)).

3.2 Stability in Water

Concentration:	No Data
Half-life:	An equivalent environmental half-life would be > 1 year at pH

4, 7, and 9.

% Hydrolyzed: < 10% degraded after 5 days at pH 7 and 50°C. A preliminary study showed that after 5 days at pH 4, 7, and 9 and at 50°C, < 10% hydrolysis had occurred. Adiponitrile is hydrolytically stable under acid, neutral, and basic conditions.

Method: Directive 84/449/EEC. C.10 "Abiotic degradation: Hydrolysis as a function of pH"

GLP: Yes

Reference: Rhone-Poulenc (1994). "Adiponitrile: Determination of hydrolysis as a function of pH," Pharmaco LSR Report No. 94/RHM007/0349 (cited in IUCLID (2000). IUCLID Data Sheet "adiponitrile" (February 18)).

Reliability: High because a scientifically defensible or guideline method was used.

Additional References for Stability in Water: None Found.

3.3 Transport (Fugacity):

Media: Air, Water, Soil, Sediments

Distributions:

Air:	0.037%
Water:	30.2 %
Soil:	69.6%
Sediment:	0.136%

Adsorption: Not Applicable

Coefficient:

Desorption: Not Applicable

Volatility: Not Applicable

Method: Calculated according to Mackay, Level III, Syracuse Research Corporation Epiwin Version 3.05. Emissions (1000 kg/hr) to air, water, and soil compartments using standard EPA model defaults.

Data Used:

Molecular Weight: 108.14

Henry's Law Constant: $1.21\text{E-}9 \text{ atm-m}^3/\text{mole}$ (Henry database)

Vapor Pressure: $6.8\text{E-}4 \text{ mm Hg}$ (Daubert and Danner, 1989)

Log Kow : -0.32 (Kowwin program)

Soil Koc : 0.196 (Log Kow estimate)

GLP: Not Applicable

Reference: Daubert, T. E. and R. P. Danner (1989). Physical and Thermodynamic Properties of Pure Chemicals Data Compilation, Taylor and Francis, Washington, DC (HSDB/627)

Syracuse Research Corporation EPIWIN v3.05 contains a

Level III fugacity model. The methodology and programming approach was developed by Dr. Donald Mackay and co-workers which is detailed in:

Mackay, D. (1991). Multimedia Environmental Models; The Fugacity Approach, pp. 67-183, Lewis Publishers, CRC Press.

Mackay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1618-1626.

Mackay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1627-1637.

Reliability: Estimated value based on accepted model.

Additional References for Transport (Fugacity): None Found.

3.4 Biodegradation

Value: Inherently biodegradable (biodegradation was 53-66% after 28 days)

Breakdown 53-66% NH₃

Products: 35-43% NO₂

Method: Directive 84/449/EEC, C.7 "Biotic degradation – modified MITI test." The test was an aerobic test using activated sludge as the inoculum (30 mg/L) and 100 mg/L of the test substance.

GLP: Unknown

Reference: Chemicals Inspection and Testing Institute (1982). Data on Existing Chemicals based on the CSCL Japan, October (cited in IUCLID (2000). IUCLID Data Sheet "adiponitrile" (February 18)).

Reliability: High because a scientifically defensible or guideline method was used.

Value: Adiponitrile had a 5-day theoretical BOD of 40% in a river die-away study using unacclimated Ohio River water, 0.5-10 mg/L substrate concentration and sewage inocula. A 12-day theoretical BOD was >100%. Negligible degradation was observed after 2 days. At a substrate concentration of 40 mg/L and 20°C, adiponitrile had theoretical CO₂ evolutions of 10 and 60% after 2 and 9 days, respectively. At 5°C, theoretical CO₂ evolution was 10 and 60% after 7.5 and 33 days, respectively. At 20°C effects of acclimation were examined by redosing, at an initial substrate concentration of 40 mg/L; the ratio of time it took to achieve 60% oxidation on the 1st and 2nd feeding was 2.1 to 1 (Ludzack et al., 1959).

	If released to water, aerobic biodegradation may be an important removal process. Results of one biodegradation screening study indicate that in unacclimated river water at 20°C, the half-life of adiponitrile is about 1 week. Acclimation of microorganisms should increase the rate of biodegradation and lower temperatures should decrease the rate of biodegradation (Ludzack et al., 1959).
Breakdown	No Data
Products:	
Method:	No Data
GLP:	No Data
Reference:	Ludzack, F. J. et al. (1959). <u>Sewage and Ind. Wastes</u> , 31:33-44 (HSDB/627).
Reliability:	Not assignable because limited study information was available.

Additional References for Biodegradation:

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Ludzack, F. J. et al. (1960). J. Water Pollut. Control Fed., 32(11):1173-1200.

Ryerman, D. W. et al. (1966). Behavior of Organic Chemicals in the Aquatic Environment, MCA, Washington, DC (cited in EPA OHM-TADS Report No. 72T16576).

Oil and Hazardous Materials, Technical Assistance Data System (ed. NIH-EPA), in Dangerous Properties of Industrial Materials Report, Nov/Dec 1987 (cited in IUCLID (2000). IUCLID Data Sheet "adiponitrile" (February 18)).

Ludzack, F. J. et al. (1961). J. Water Pollut. Control Fed., 33:492-505 (BIODEG/103446 and 103447).

Ludzack, F. J. et al. (1959). Proc. of the 14th Industrial Waste Conf., Eng. Ext. Ser. 104:547-65 (HSDB/627).

Lutin, P. A. (1970). J. Water Poll. Contr. Fed., 42:1632-42 (HSDB/627).

Data from this additional source were not summarized because the test substance was a mixture or otherwise inappropriate.

Monsanto Co. (1983). Preliminary Studies on the Degradation of Nitriles by Brevibacterium SP R-312, TSCA Fiche OTS0515380.

3.5 Bioconcentration

Value:	BCF <1. This BCF value suggests that this compound will not bioaccumulate significantly in aquatic organisms (SRC, n.d.).
Method:	Estimated using linear regression equations based on a measured log Kow of -0.32 and a measured water solubility of 8×10^4 mg/L at 20°C (Smiley, 1981; Tanii and Hashimoto, 1985; Lyman et al., 1982; SRC, n.d.).
GLP:	Not Applicable
Reference:	Smiley, R. A. (1981). <u>Kirk-Othmer Encycl. Chem. Tech.</u> , 3 rd ed., 15:897-901, John Wiley and Sons, New York (HSDB/627). Tanii, H. and K. Hashimoto (1985). <u>Arch. Toxicol.</u> , 57:88-93 (HSDB/627). Lyman, W. J. et al. (1982). <u>Handbook of Chemical Property Estimation Methods</u> , p. 5-5, McGraw-Hill, New York (HSDB/627). SRC (Syracuse Research Corporation) (n.d.). (HSDB/627).
Reliability:	Estimated value based on acceptable model.

Additional References for Bioconcentration: None Found.

4.0 Ecotoxicity

4.1 Acute Toxicity to Fish

Type:	96-hour LC₅₀
Species:	<i>Pimephales promelas</i> (fathead minnow)
Value:	1930 mg/L (confidence limits, 1850 – 2020 mg/L)
Method:	Fathead minnows used in the tests were cultured from brood stock provided by the U.S. EPA Environmental Research Laboratory-Duluth. Fish that were approximately 30 days old were used in the toxicity test. Fish were not fed during chemical exposures. Test concentrations were 0, 477, 734, 1130, 1740, and 2670 mg/L.

During the flow-through exposure, the fish were routinely observed for behavioral responses (effects) and death. Death was defined as the cessation of opercular movements and the inability to respond when prodded. Dead fish were removed and recorded at 3, 6, 12, 24, 48, 72, and 96 hours from initial exposure.

Five water quality parameters were routinely measured for

each test. Results of these tests indicated a water temperature of 26.2°C, dissolved oxygen of 6.2 mg/L, hardness of 45.5 mg/L CaCO₃, alkalinity of 41.5 mg/L CaCO₃, and pH 7.6. Analytical monitoring was conducted using gas-liquid chromatography.

Test exposure chambers were sampled at approximately mid-depth at 0, 24, 48, 72, and 96 hours in all exposure chambers.

GLP: No
Test Substance: Adiponitrile, purity 99%
Results: The affected fish became excitable, stayed near the water surface with rapid fin and opercular movements.
Reference: Brooke, L. T. et al. (1984). Acute Toxicities of Organic Chemicals to Fathead Minnow (*Pimephales promelas*), Vol. I, pp. 3-13, 157-158, Center for Lake Superior Environmental Studies, University of Wisconsin Superior.
Reliability: High because a scientifically defensible or guideline method was used.

Additional References for Acute Toxicity to Fish:

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Monsanto Co. (1981). Analytical Bio Chemistry Laboratories Report No. 27351, April 22, TSCA Fiche OTS0514857.

Monsanto Co. (1981). Analytical Bio Chemistry Laboratories Report No. 27352, May 6, TSCA Fiche OTS0514858.

Henderson, C. et al. (1960). Purdue Univ. Eng. Bull., Ext. Ser., (106):120-130.

DuPont Co. (1975). Unpublished Data, Haskell Laboratory Report No. 116-75 (also cited in TSCA Fiche OTS0514973).

Knie, J. et al. (1983). Deutsche Gewaesserkundliche Mitteilungen, 27, 3:77-79 (cited in IUCLID (2000). IUCLID Data Sheet "adiponitrile" (February 18)).

Data from these additional sources were not summarized because the study design was not adequate.

Eldred, D. V. et al. (1999). Chem. Res. Toxicol., 12:670-678.

Kaiser, K. L. E. et al. (1997). Water Qual. Res. J. Can., 32(4):855

(CA127:273987).

Cronin, M. T. D. et al. (1991). Sci. Total Environ., 109-110:431-439
(CA116:122786).

4.2 Acute Toxicity to Invertebrates

Type:	48-hour EC ₅₀
Species:	<i>Daphnia magna</i> (water flea)
Value:	>1000 mg/L
Method:	The procedures for static bioassay, as described in American Public Health Association, 1975 and Stephan, 1975 were used in the experiment. The <i>Daphnia magna</i> used in the test were cultured at the ABC facilities. The adult <i>Daphnia</i> were fed a suspension of trout chow and alfalfa daily until 24 hours prior to testing.
	The bioassay was conducted in 250 mL glass beakers kept at 20±1.0°C. The photoperiod was controlled to give 16 hours daylight and 8 hours darkness.
	Five concentrations in duplicate of the test compound with 10 <i>Daphnia</i> (first instar less than 24 hours old) per beaker were selected for their respective bioassay. The concentrations tested were 0, 100, 180, 320, 560, and 1000 mg/L.
GLP:	Yes
Test Substance:	Adiponitrile, purity not specified
Results:	The water chemistry characteristics are presented in the following table.

Concentration (mg/L)	Temperature (°C)	Dissolved Oxygen (mg/L)	pH
0	20a 21	8.8a 7.8	8.6a 8.6
100	21	4.3	8.4
180	---	---	---
320	21	8.0	8.7
560	---	---	---
1000	21	8.2	8.7
a = Measurement at 0 hours. All other measurements were done at 48 hours. --- = No data were reported.			

Reference: The no-effect level observed for adiponitrile was 320 mg/L after 48 hours.
Monsanto Co. (1981). Analytical Bio Chemistry Laboratories Report No. 27357, April 24, TSCA Fiche OTS0514860.

American Public Health Association (1975). Standard Methods for the Examination of Water and Wastewater, 14th ed., New York (cited in Monsanto, 1981).

Reliability: Stephan, C. E. (1975). Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians, Committee on Methods for Toxicity Tests with Aquatic Organisms, U.S. EPA, Ecol. Res. Ser. 660/3-75009 (cited in Monsanto, 1981).
Medium because a suboptimal study design was used that did not include measurement of test substance concentrations during the study.

Additional References for Acute Toxicity to Invertebrates:

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Bringmann, G. and R. Kuehn (1982). Z. Wasser. Abwasser Forsch., 15(1):1-6.

Knie, J. et al. (1983). "Results of Studies on Chemical Substances with Four Biotests," Dtsch. Gewaesserkd. Mitt., 27(3):77-79 (cited in IUCLID (2000). IUCLID Data Sheet "adiponitrile" (February 18)).

Data from this additional source were not summarized because the study design was not adequate.

Cronin, M. T. D. et al. (1991). Sci. Total Environ., 109-110:431-439 (CA116:122786).

4.3 Acute Toxicity to Aquatic Plants

Type:	72-hour biomass and growth rate test
Species:	<i>Selenastrum capricornutum</i> (algae)
Value:	NOEC >100 mg/L
Method:	Directive 87/302/EEC, Part C, "Algal inhibition test". Algal cultures (6 replicates) with an initial cell count of 10^4 per mL were exposed to adiponitrile at a nominal concentration of 100 mg/L in mineral salts medium or to mineral salts medium alone. The cell density of each culture was measured using a hemocytometer at 24-hour intervals. The concentration of the test substance in the medium was analyzed.
GLP:	Yes
Test Substance:	Adiponitrile, purity not specified
Results:	Results for replicate samples at the beginning and end of the test indicated that the intended exposure concentration was achieved and adequately maintained. The overall mean measured level was 97.4 mg/L.
Reference:	Exposure of <i>Selenastrum capricornutum</i> at a limit concentration of 100 mg/L did not result in a significant reduction in either the average specific growth rate or biomass of test cultures compared to control cultures. Rhone-Poulenc (1994). "Adiponitrile: Determination of 72-hour EC ₅₀ to <i>Selenastrum capricornutum</i> ," Pharmaco LSR Report No. 94/RHM008/0311 (cited in IUCLID (2000). IUCLID Data Sheet "Adiponitrile" (February 18)).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Acute Toxicity to Aquatic Plants: None Found.

5.0 Mammalian Toxicity

5.1 Acute Toxicity

Type:	Oral LD ₅₀
Species/Strain:	Male rats/Crl:CD®
Value:	Fasted rat: 138 mg/kg (confidence limits, 122-156 mg/kg) Non-fasted rat: 301 mg/kg (confidence limits, undefined)
Method:	The test substance, as a suspension in corn oil, was administered via intragastric intubation in single doses to 4 groups of fasted and 6 groups of non-fasted young adult male rats. Dose levels of 100, 130, 150, and 200 mg/kg were used for the fasted rats and dose levels of 250, 300, 325, 340, 370, and 400 mg/kg were used for the non-fasted rats. Ten rats were used for each dose level tested. The surviving rats were weighed and observed during a 14-15 day recovery period and then sacrificed. LD ₅₀ calculations were done according to the method of D. J. Finney.
GLP:	No
Test Substance:	Adiponitrile, purity not specified
Results:	Mortality occurred in 0/10, 5/10, 7/10, and 9/10 rats in the 100, 130, 150, and 200 mg/kg fasted dose groups, respectively. Mortality occurred in 3/10, 7/10, 5/10, 3/10, 8/10, and 10/10 rats in the 250, 300, 325, 340, 370, and 400 mg/kg non-fasted dose groups, respectively. Clinical signs observed at most dose levels included stained and wet perineal area, stained face, diarrhea, weakness, and weight loss. All deaths occurred within 3 days after dosing.
References:	DuPont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 23-81 (also cited in TSCA Fiche <u>OTS0514991</u>). Dashiell, O. L. and G. L. Kennedy, Jr. (1984). <u>J. Appl. Toxicol.</u> , 4(6):320-325.
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Acute Oral Toxicity:

Data from these additional sources support the study results summarized above. The studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Dieke, S. H. et al. (1947). J. Pharmacol. Exp. Ther., 90:260-270.

Plokhova, E. I. and A. P. Rubakina (1965). Gig. Tr. Prof. Zabol., 9(9):56-58 (CA64:2650h).

DuPont Co. (1975). Unpublished Data, Haskell Laboratory Report No. 124-75 (also cited in TSCA Fiche OTS0514974).

DuPont Co. (1950). Unpublished Data, Haskell Laboratory Report No. 20-50 (also cited in TSCA Fiche OTS0514976).

DuPont Co. (1982). Unpublished Data, Haskell Laboratory Report No.131-82 (also cited in TSCA Fiche OTS0514994).

Kennedy, G. L., Jr. et al. (1986). J. Appl. Toxicol., 6(3):145-148.

Svirbely, J. L. and E. P. Floyd (1964). Toxicologic Studies of Acrylonitrile, Adiponitrile, and β , β' -oxydipropionitrile III. Chronic Studies, U.S. D.H.E.W., PHS, Bureau of State Services, Robert A. Taft Sanitary Engineering Center.

Szabo, S. et al. (1982). J. Pharmacol. Exp. Ther., 223(1):68-76.

Monsanto Co. (1969). Younger Laboratories Report, TSCA Fiche OTS0516563.

Anon. (1984). Gig. Sanit., 49(12):40 (RTECS/40256).

Klimkina, N. V. and E. S. Bruk (1967). Prom. Zag. Vod., (8):85-100 (CA69:38567c).

Tanii, H. and K. Hashimoto (1985). Arch. Toxicol., 57(2):88-93.

Anon. (1969). Zentralbl. Arbeitsmed. Arbeitsschutz, 19:225 (RTECS/40256).

Ceresa, C. (1948). Med. Lav., 39:162-165 (CA43:1985g).

Ghiringhelli, L. (1955). Med. Lav., 46:221-228 (cited in IUCLID (2000). IUCLID Data Sheet "adiponitrile" (February 18)).

Type:	Inhalation LC₅₀
Species/Strain:	Male rats/ChR-CD [®]
Exposure Time:	4-hour
Value:	1.71 mg/L
Method:	The test substance was syringe driven into a stainless steel tube that was installed in a Lindberg furnace. The furnace was heated to a temperature sufficient to vaporize the test material (260-305°C). Air was passed through the stainless steel tube at measured rates to force the vaporized material into the exposure chamber. Atmospheric concentrations were determined spectrophotometrically. Samples of test atmosphere

	were collected at approximately 30-minute intervals. Groups of 10 male rats were exposed to the vapor in a 20 L glass chamber for single 4-hour exposures at concentrations of 0.25, 0.36, 0.71, 0.83, 1.18, 1.95, 2.47, 2.53, 2.70, and 3.03 mg/L. Following each exposure, surviving rats were weighed and observed for clinical signs daily (weekends excluded) for a 14-day recovery period.
GLP:	No
Test Substance:	Adiponitrile, purity not specified
Results:	Mortality occurred in 0/10, 0/10, 5/10, 0/10, 6/10, 7/10, 9/10, 8/10, 6/10, and 7/10 rats in the 0.25, 0.36, 0.71, 0.83, 1.18, 1.95, 2.47, 2.53, 2.70, and 3.03 mg/L groups, respectively. Clinical signs observed during exposures were labored breathing, salivation, lethargy, red ears, gasping, and convulsions. Post-exposure observations included lethargy, lung noise, weight loss, and wet perineal areas. In the absence of a linear trend, the 4-hour LC ₅₀ was estimated to be 1.71 mg/L.
Reference:	DuPont Co. (1979). Unpublished Data, Haskell Laboratory Report No. 394-79 (also cited in TSCA Fiche <u>OTS0540602</u> and <u>OTS0516027</u>). Kennedy, G. L., Jr. and L. W. Smith (1981). <u>The Toxicologist</u> , 1(1):76 (Abstract No. 275). Smith, L. W. and G. L. Kennedy (1982). <u>Toxicol. Appl. Pharmacol.</u> , 65(2):257-262.
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Acute Inhalation Toxicity:

Data from these additional sources support the study results summarized above. The studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Environmental Health Laboratory (1979). Project 780065, ML-78-341, TSCA Fiche OTS0514859.

DuPont Co. (1975). Unpublished Data, Haskell Laboratory Report No. 216-75 (also cited in TSCA Fiche OTS0514988).

Monsanto Co. (1982). Report No. MSL-2267, May 24, TSCA Fiche OTS0515371.

DuPont Co. (1950). Unpublished Data, Haskell Laboratory Report No. 20-50

(also cited in TSCA Fiche [OTS0514976](#)).

DuPont Co. (1950). Unpublished Data, Haskell Laboratory Report No. 21-50
(also cited in TSCA Fiche [OTS0514977](#)).

Type:	Dermal LD₅₀
Species/Strain:	Male rabbits/New Zealand White
Exposure Time:	24 hours
Value:	2134 mg/kg
Method:	Groups of 6 adult male rabbits were clipped free of hair over the back and trunk area and fitted with plastic collars. The test material (1000, 1250, 1350, 1500, 2000, or 4000 mg/kg) was applied to intact skin on each rabbit under one gauze pad. The trunk of each rabbit was then wrapped with a layer of plastic wrap, stretch gauze bandage, and elastic adhesive tape. After a 24-hour exposure period, the wrappings were removed, the rabbits were wiped with a dry towel, and returned to their cages. The rabbits were observed and/or weighed daily (except weekends) over a 14-day recovery period and then sacrificed.
GLP:	No
Test Substance:	Adiponitrile, purity not specified
Results:	Mortality of 0/6, 0/6, 3/6, 4/6, 3/6, 4/6 was observed in the 1000, 1250, 1350, 1500, 2000, and 4000 mg/kg dose groups, respectively. Intermittent weight loss occurred at all levels tested. Gasping, diarrhea, and lethargy occurred at 4000 mg/kg. All deaths occurred within 2 days after dosing.
Reference:	DuPont Co. (1982). Unpublished Data, Haskell Laboratory Report No. 532-82.
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Acute Dermal Toxicity:

Data from these additional sources support the study results summarized above. The studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Monsanto Co. (1969). Younger Laboratories Report, TSCA Fiche [OTS0516563](#) and [OTS0546110](#).

DuPont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 51-81
(also cited in TSCA Fiche [OTS0514992](#) and [OTS0555320](#)).

DuPont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 11-81.

10-July-2001

DuPont Co. (1982). Unpublished Data, Haskell Laboratory Report No. 131-82 (also cited in TSCA Fiche OTS0514990).

DuPont Co. (1975). Unpublished Data, Haskell Laboratory Report No. 449-75 (also cited in TSCA Fiche OTS0514989 and OTS0555320).

Data from this additional source were not summarized because insufficient study information was available.

Anon. (1969). Zentralbl. Arbeitsmed. Arbeitsschutz, 19:225 (RTECS/40256).

Type:	Dermal Irritation
Species/Strain:	Male rabbits/Albino
Method:	Six male rabbits were clipped free of hair on the trunk and lateral areas and placed in FDA-type stocks. Doses of 0.5 mL of undiluted test substance were applied to intact skin under gauze adhesive tape (double thickness). After 24 hours, the rabbits were removed from the stocks, the patches taken off, and the reactions observed. Observations were also made at 48 hours.
GLP:	No
Test Substance:	Adiponitrile, purity not specified
Results:	No skin reactions were observed 24 or 48 hours after treatment.
Reference:	DuPont Co. (1973). Unpublished Data, Haskell Laboratory Report No. 142-73.
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Acute Dermal Irritation:

Data from these additional sources support the study results summarized above. The studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Monsanto Co. (1969). Younger Laboratories Report, TSCA Fiche OTS0516563.

DuPont Co. (1947). Unpublished Data, Haskell Laboratory Report No. 54-47 (also cited in TSCA Fiche OTS0555808 and OTS0514975).

Zeller et al. (1969). Zentralbl. Arbeitsmed. Arbeitsschutz, 19(8):225-238 (cited in IUCLID (2000). IUCLID Data Sheet "adiponitrile" (February 18)).

Data from this additional source were not summarized because the focus of the study was skin corrosion.

Monsanto Co. (1972). Younger Laboratories Report, TSCA Fiche OTS0516564.

Type: **Dermal Sensitization**
Species/Strain: Guinea pig/Strain not specified
Method: 10 guinea pigs were tested. No additional information on method was reported.
GLP: No
Test Substance: Adiponitrile, purity not specified
Results: Negative responses were recorded in all guinea pigs in the irritation and sensitization phases of the test.
Reference: DuPont Co. (1947). Unpublished Data, Haskell Laboratory Report No. 54-47 (also cited in TSCA Fiche OTS0555808 and OTS0514975).
Reliability: Medium because a suboptimal study design was used and insufficient study information was available.

Additional References for Acute Dermal Sensitization: None Found.

Type: **Eye Irritation**
Species/Strain: Male and female rabbits/Albino
Method: 0.1 mL of undiluted sample of the test substance was placed in the conjunctival sac of the right eye of each of 3 albino male and female rabbits. Observations were made over a period of several days for inflammation. The eyes were rinsed with warm isotonic saline solution after 24 hours.
GLP: No
Test Substance: Adiponitrile (refined), purity not specified
Results: Adiponitrile was a slight eye irritant in male and female rabbits. The average maximum score was 10.6/110 in 1 hour. There was moderate discomfort with blinking immediately after application and shortly after the lids were closed. In 10 minutes, there was barely perceptible redness, slight to mild edema, and mild discharge. In 1 hour, erythema and edema remained the same; discharge was moderate with slight whitish exudate. There was improvement after irrigation so that within 48 hours, the edema disappeared, discharge ceased, and only barely perceptible redness remained in 2/3 rabbits. All rabbits received a score of 0 within 3 days.
Reference: Monsanto Co. (1969). Younger Laboratories Report, Monsanto Project No. Y-69-43, TSCA Fiche OTS0516563.
Reliability: High because scientifically defensible or guideline method was used.

Additional Reference for Acute Eye Irritation:

Data from this additional source support the study results summarized above. The study was not chosen for detailed summarization because the data were not substantially additive to the database.

Zeller, H. et al. (1969). Zentral Bl. Arbeitsmed. Arbeitsschutz, 19(8):225-238 (cited in IUCLID (2000). IUCLID Data Sheet "adiponitrile" (February 18)).

5.2 Repeated Dose Toxicity

Study No. 1

Type: 13-Week Inhalation Study in Rats

Species/Strain: Rats/Sprague-Dawley

Sex/Number: Male and female/15 per concentration per sex

Exposure

Period: 13 weeks (total of 66 exposure days)

Frequency of

Treatment: 6 hours/day, 5 days/week

Exposure

Levels: 0, 12.9, 30.6, 99 mg/m³

Method: Rats were 46-51 days of age at study start. Animal rooms were maintained routinely at 72±2°F and 35-60% relative humidity. Food and water were available *ad libitum*.

Rochester-style inhalation chambers, 10 m³, were used for the exposures. The test substance for the 30.6 and 99 mg/m³ groups was metered from a tank using a capillary restrictor to a nebulizer which was used to generate the test atmosphere. The test substance for the 12.9 mg/m³ group was delivered using a syringe pump to the nebulizer. The concentration of the test substance was controlled either by regulating the pressure in the tank headspace or in the syringe pump. Sampling of the test chamber atmosphere occurred 4 times per exposure day for the first 27 days and at least twice per exposure thereafter. The concentration was determined by gas chromatography. Uniformity of distribution of the adiponitrile vapor and aerosol was also determined. Particle size of the aerosol was determined in the high concentration group.

Body weights and clinical observations of the rats were recorded periodically throughout the study. Blood and urine examinations were performed on a total of 15 rats/sex/group. The blood and urine were obtained from 5 pre-selected rats/sex/group on each of the mornings following 2 days of exposure common to all rats during the last week of the study.

The animals had been fasted for approximately 22 hours prior to the blood collection. The urine specimens were collected during the 15 hours immediately following the last exposure. Nine hematology parameters and 14 blood chemistry parameters were measured or calculated. Urinalysis included thiocyanate measurements.

Five pre-selected, fasted rats/sex/group underwent necropsy on 3 designated necropsy days. The nasal, cranial, thoracic, abdominal, and scrotal cavities were opened and contents were removed and examined. Approximately 33 tissues were removed and prepared for histological examination. Seven of these tissues were weighed. The tissues from the high and control groups were histologically examined.

GLP:

Yes

Test Substance:

Adiponitrile, purity 99%

Results:

The vapor and aerosol were determined to be uniformly distributed throughout the chamber. Mean chamber environmental measurements of airflow, temperature, and humidity were between 1727-1782 L/min, 23.3-25.3°C, and 48.5-49.2%, respectively. The mass medium aerodynamic diameter (MMAD) of aerosol particles less than 10 microns at 99 mg/m³ was determined to be between 3.46 and 4.19 microns. There was predominantly aerosol concentration of adiponitrile in the high exposure chamber.

No gross signs of toxicity related to test substance exposure were noted and no deaths occurred during the study. No statistically significant changes were observed at any time during the study in mean body weights.

Females at 99 mg/m³ had mildly decreased red blood cells, hemoglobin, and hematocrit levels. Males and females at all levels had increased urine thiocyanate values. Significant necropsy findings were non-existent and there were no compound-related microscopic lesions.

No toxicological effect of adiponitrile aerosol and vapor was observed at 30.6 mg/m³ and below.

Reference:

Monsanto Co. (1983). Report No. MSL-2937, April 18, TSCA Fiche OTS0515373.

Short, R. D. et al. (1990). J. Toxicol. Environ. Health, 30(3):199-207.

Reliability:

High because a scientifically defensible or guideline method was used.

Study No. 2

Type: 2-Year Drinking Water Study in Rats

Species/Strain: Rats/CFR

Sex/Number: Male and female/25 per concentration per sex

Exposure

Period: 2 years

Frequency of

Treatment: Not specified

Exposure

Levels: 0, 0.5, 5, 50 ppm

Method: Body weights and water consumption were measured in the study. Periodic hematological observations (hematocrit, hemoglobin, white blood cells, differential count) were performed. Routine histopathological examination on a representative number of rats occurred at the end of the study. Organ weights and organ/body weight ratios were recorded for spleen, liver, and kidney.

GLP: No

Test Substance: Adiponitrile, purity not specified

Results: Mortality ratios of 11/25, 7/25, and 5/25 occurred for 0.5, 5.0, and 50.0 ppm male rats, respectively. Mortality ratios of 7/25, 8/25, and 5/25 for 0.5, 5.0, and 50.0 ppm occurred for the female rats, respectively.

Body weight, water consumption, hematological values, and organ/body weight ratios were not affected. Advanced adrenal degeneration was found in female rats exposed at all 3 concentrations of adiponitrile and in males exposed at 50 ppm. Degeneration of other organs was noted, but was attributed to the aging of the rats.

Reference: Svrbely, J. L. and E. P. Floyd (1964). Toxicologic Studies of Acrylonitrile, Adiponitrile, and β , β' -oxydipropionitrile III. Chronic Studies, U.S. D.H.E.W., PHS, Bureau of State Services, Robert A. Taft Sanitary Engineering Center.

Reliability: Not assignable because limited study information was available.

Additional References for Repeated Dose Toxicity:

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Anon. (1981). Gig. Tr. Prof. Zabol., 25(9):64 (RTECS/AV2625000).

- Ceresa, C. (1948). Med. Lav., 39:162-165 (CA43:1985g).
- Dieke, S. H. et al. (1947). J. Pharmacol. Exp. Ther., 90:260-270.
- DuPont Co. (1950). Unpublished Data, Haskell Laboratory Report No. 20-50 (also cited in TSCA Fiche OTS0514976 and OTS0590002).
- DuPont Co. (1950). Unpublished Data, Haskell Laboratory Report No. 21-50 (also cited in TSCA Fiche OTS0514977).
- DuPont Co. (1979). Unpublished Data, Haskell Laboratory Report 747-79 (also cited in TSCA Fiche OTS0514987).
- Ghiringhelli, G. L. (1956). Med. Lav., 47:192-199 (CA51:1461d).
- Kennedy, G. L., Jr. and L. W. Smith (1981). The Toxicologist, 1(1):76 (Abstract No. 275).
- Klimkina, N. V. and E. S. Bruk (1967). Prom. Zag. Vod., (8):85-100.
- Monsanto Co. (1982). Report No. MSL-2593, October 19, TSCA Fiche OTS0515372.
- Monsanto Co. (1984). Report No. MSL-3540, May 3, TSCA Fiche OTS0515370.
- Monsanto Co. (1984). Report No. MSL-3416, February 16, TSCA Fiche OTS0514855.
- Plokhova, E. I. and A. P. Rubakina (1965). Gig. Tr. Prof. Sabol., 9:56-58.
- Smith, L. W. and G. L. Kennedy, Jr. (1982). Toxicol. Appl. Pharmacol., 65(2):257.
- Svirbely, J. L. and E. P. Floyd (1964). Toxicologic Studies of Acrylonitrile, Adiponitrile, and β , β' -oxydipropionitrile III. Chronic studies, U.S. D.H.E.W., PHS, Bureau of State Services, Robert A. Taft Sanitary Engineering Center.
- Szabo, S. et al. (1982). J. Pharmacol. Exp. Ther., 223(1):68-76 (CA97:209875).
- Zeller, H. et al. (1969). Zentral Bl. Arbeitsmed. Arbeitsschutz, 19(8):225-238 (cited in IUCLID (2000). IUCLID Data Sheet "adiponitrile" (February 18)).
- Data from these additional sources were not summarized because the test substance was a mixture or otherwise inappropriate.

Monsanto Co. (1984). Study No. IR-83-223, TSCA Fiche OTS0515387.

Monsanto Co. (1985). Study No. IR-83-224, TSCA Fiche OTS0515385 and OTS0515386.

5.3 Developmental Toxicity

Species/Strain: Rats/Charles River COBS[®] CD[®]
Sex/Number: Female/25 per group
Route of Administration: Gavage
Exposure Period: Gestation days 6 – 19
Frequency of Treatment: Daily
Exposure Levels: 0, 30, 50, 80 mg/kg
Method: Rats were approximately 14 weeks old at the time of mating. One male and 1 female rat were placed together for mating. The occurrence of copulation was determined by daily inspection for a copulatory plug or by a vaginal smear for sperm. The day that evidence of mating was detected was designated day 0 of gestation and the female was returned to an individual cage.

The test substance was mixed daily with the vehicle, Mazola[®] corn oil. Observations for mortality and behavior as well as body weights were periodically recorded throughout the study. Any animals that died prior to the scheduled sacrifice underwent a gross necropsy and any developing fetuses were examined externally. On gestation day 20, all surviving dams were sacrificed. Immediately following sacrifice, the uterus was excised and weighed prior to removal of the fetuses. The number and location of viable and nonviable fetuses, early and late resorptions, and the number of total implantations and corpora lutea were recorded. The abdominal and thoracic cavities and organs of the dams were examined for grossly evident morphological changes. All fetuses were individually weighed and examined for external malformations and variations, including the palate and eyes. Each fetus was externally sexed. Approximately half of the fetuses were placed in Bouin's fixative for subsequent visceral examination by razor blade sectioning as described by Wilson. The remaining half of the fetuses underwent skeletal examination.

GLP: Yes
Test Substance: Adiponitrile, purity not specified

Results: Survival was 100% in the control and 30 mg/kg groups. One rat in the 50 mg/kg/day group died on gestation day 17; cause of death could not be determined at autopsy. Two females in the 80 mg/kg/day group died prior to the scheduled sacrifice; one each on days 9 and 20. At necropsy, severe lung congestion was noted in one dam and peritonitis and pericarditis were seen in the other dam.

There were no biologically meaningful differences in appearance or behavior, mean maternal body weight gains, the mean numbers of corpora lutea, total implantations, post-implantation loss, viable fetuses, the fetal sex distribution, or mean fetal body weight in any of the treated groups compared to controls. A summary of some of the reproductive outcomes (means/litter) are provided in the tables below:

Dosage (mg/kg)	0	30	50	80
Corpora lutea:	14.9	17.0	15.6	16.4
Implantations:	13.5	15.0	13.5	14.4
Postimplantation Loss:	0.7	1.5	0.8	0.9
Total No. of Fetuses:	12.7	13.5	12.8	13.5
Total No. of Live Fetuses:	12.7	13.5	12.8	13.5
Mean Fetal Weight (g):	3.6	3.4	3.7	3.4
Sex Ratio (No. of Males/Total No.):	0.54	0.54	0.52	0.47

There were no biologically meaningful or statistically significant differences in the number of litters with malformations. The number of fetuses or litters with genetic or developmental variations in the treated groups were also comparable to the control group.

Treatment with adiponitrile did not produce a teratogenic response at doses \leq 80 mg/kg.

Reference: Monsanto Co. (1981). International Research and

Development Corp. Project No. IR-79-166, January 27, TSCA Fiche OTS0514854.

Johannsen, F. R. et al. (1986). Fundam. Appl. Toxicol., 7(1):33-40.

Johannsen, F. R. et al. (1986). Fundam. Appl. Toxicol., 7(4):690-697.

Reliability: High because a scientifically defensible or guideline method was used.

Additional References for Developmental Toxicity:

Data from this additional source supports the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

International Research and Development Corp. (1980). December 19, TSCA Fiche OTS0514853.

Data from this additional source were not summarized because the study design was not adequate.

Gombar, V. K. et al. (1991). Quant. Struct.-Act. Relat., 10(4):306-332 (CA117:2498).

5.4 Reproductive Toxicity

Study No. 1

Species/Strain: Rats/Crl:CD[®](SD)BR

Sex/Number: Female/24 per group
Male/15 per group

Route of Administration: Inhalation

Exposure Period: 22 days prior to mating

Frequency of Treatment: 6 hours/day, 7 days/week

Exposure Levels: 0, 13, 30, 100 mg/m³

Method: Female rats were exposed by inhalation for 22 days and mated to untreated males to assess female fertility. Exposure of the females was continued until copulation was confirmed. Female rats were 73 days of age on the first day of exposure. Male rats were 83 days of age at mating. Food and water were available *ad libitum* except during the inhalation exposures for

the females. Males were not exposed to the test substance.

Inhalation exposures were conducted in 10 m³ Rochester-style stainless steel and glass inhalation chambers. Atmospheres were produced by metering the liquid through a nebulizer. Concentrations of the test substance in the exposure chambers were measured by gas chromatographic analysis. Nominal concentration measurements, particle-size measurements, temperature, and humidity measurements were also conducted for the exposure chambers. Observations of the animals for signs of toxicity occurred during the exposure.

Body weights were periodically measured throughout the study. Females were given a thorough physical examination once per week and were observed for clinical signs before and after exposure. All animals were checked twice daily for mortality and gross abnormalities. Vaginal smears were taken on 5 consecutive days for unmated females.

Females were mated to an untreated male of the corresponding group until copulation was confirmed or 5 nights occurred. Copulation was confirmed by the presence of a copulatory plug on the cageboard or vaginal smears indicated the presence of sperm. Females which failed to mate at the initial mating were assigned to a proven male in the same group until copulation was confirmed or 7 nights had elapsed.

Females were sacrificed at mid-gestation (gestation days 13-15). Females without confirmed copulation were sacrificed in the second week after the last day of co-housing. Each female was given an external examination prior to sacrifice. Tissues and organs of the thoracic and abdominal cavities were examined for gross lesions, pregnancy status was determined and, for pregnant females, nidation sites were classified and counted, and corpora lutea were counted. Male rats were sacrificed after mating and no necropsies were performed.

GLP: Yes

Test Substance: Adiponitrile, purity 99%

Results: The average mean analytical exposure concentrations were 13.0, 31.8, and 104 mg/m³ for the 13, 30, and 100 mg/m³ groups, respectively. Particle-size measurements indicated a mass median aerodynamic diameter of between 3.53 and 4.19 µm. Adiponitrile was predominantly present as an aerosol at the 100 mg/m³ level. Temperature and humidity measurements indicated no extreme conditions during

exposure with mean temperatures of 24-26°C and mean percent relative humidities of 41-46%.

No toxic effects were seen in any treated females other than slightly lower body weights of 100 mg/m³ females in the 2nd and 3rd weeks of exposure. There were no deaths of any females prior to sacrifice. There were no treatment related adverse clinical signs or lesions detected at gross necropsy. There were also no significant detectable effects on female fertility at any dose level tested. Treatment group females were comparable to controls in mating efficiency, pregnancy rate, number of live implants, and pre- and post-implantation loss rates.

Reference: Monsanto Co. (1983). Report No. MSL-2990, May 20, TSCA Fiche OTS0516561.

Short, R. D. et al. (1990). J. Toxicol. Environ. Health, 30(3):199-207.

Reliability: High because a scientifically defensible or guideline method was used.

Study No. 2

Species/Strain: Rats/Crl:CD[®](SD)BR

Sex/Number: Male/12 per group
Female/40 per group

Route of Administration: Inhalation

Exposure Period: 53 exposure days (74 days on study)

Frequency of Treatment: 6 hours/day, 5 days/week

Exposure Levels: 0, 13, 30, 100 mg/m³

Method: Male rats were exposed by inhalation for 53 days and mated to untreated virgin females to assess male fertility. Male rats were 45 days of age on the first day of exposure. Female rats were 78 days of age at mating. Food and water were available *ad libitum* except during the inhalation exposures for the males. Exposure of the males was continued until the day before sacrifice. Females were not exposed to the test substance.

Inhalation exposures were conducted in 10 m³ Rochester-style stainless steel and glass inhalation chambers. Atmospheres were produced by metering the liquid through a nebulizer. Concentrations of the test substance in the exposure chambers

were measured by gas chromatographic analysis. In addition to analytical determinations of exposure concentration, uniformity of distribution of the chamber atmospheres and particle-size distribution were also measured. Other measurements included nominal concentration, temperature, and humidity measurements. Observations of the animals for signs of toxicity also occurred during the exposure.

Body weights were periodically measured throughout the study. Males were given a thorough physical examination once per week and were observed for clinical signs before and after exposure. All animals were checked twice daily for mortality and gross abnormalities.

After sufficient time on study to cover the spermatogenesis cycle of the rat, males were mated consecutively to 3 females. Each mating continued until confirmed copulation was observed or 5 nights occurred without confirmed copulation. Copulation was confirmed by the presence of a copulatory plug on the cageboard. If the location of the plug was ambiguous, vaginal smears of the appropriate females were taken and examined for the presence of sperm.

One-third of the males of each treatment group were sacrificed on each of 3 consecutive days at the end of the study. Each male was given an external examination prior to sacrifice. Tissues and organs of the thoracic, abdominal, and scrotal cavities were examined and testes, epididymides, prostate glands, and seminal vesicles were preserved.

Females which were not co-housed with males were sacrificed and no necropsy was performed. Mated females were sacrificed at mid-gestation (gestation days 12-15) and necropsied. Females without confirmed copulation were sacrificed in the 2nd week after the last day of co-housing. Tissues and organs of the thoracic and abdominal cavities were examined for gross lesions, pregnancy status was determined, and for pregnant females, total nidations, number of resorptions, live implantations, and corpora lutea were counted.

GLP:	Yes
Test Substance:	Adiponitrile, purity 99%
Results:	The average mean analytical exposure concentrations were 12.9, 30.6, and 99 mg/m ³ for the 13, 30, and 100 mg/m ³ groups, respectively. Particle-size measurements indicated a mass median aerodynamic diameter of between 3.46 and

4.19 μm at 100 mg/m^3 . Adiponitrile was predominantly present as an aerosol at the 100 mg/m^3 level. Distribution measurements indicated uniform distribution of vapor/aerosol atmospheres within the chambers. Temperature and humidity measurements indicated no extreme conditions during exposure with mean temperatures of 23-25°C and mean percent relative humidities of 48-49%.

There were no deaths of any males prior to sacrifice. No toxic effects were seen on body weight, clinical signs, or lesions detected at gross necropsy. There were no detectable effects on male fertility at any exposure level tested. Treated males were comparable to controls in mating efficiency and efficiency in effecting pregnancies that were normal in number of live implants and pre- and post-implantation loss rates.

Reference: Monsanto Co. (1983). Report No. MSL-2989, May 20, TSCA Fiche OTS0515374.

Short, R. D. et al. (1990). J. Toxicol. Environ. Health, 30(3):199-207.

Reliability: High because a scientifically defensible or guideline method was used.

Study No. 3

Species/Strain: Rats/Sprague-Dawley (Holtzman strain)

Sex/Number: Male and female/Number not specified

Route of

Administration: Oral in the drinking water

Exposure

Period: 3 Generations

Frequency of

Treatment: Not specified

Exposure

Levels: 10, 100, 500 ppm

Method: No Data

GLP: No

Test Substance: Adiponitrile, purity not specified

Results: Reproduction studies on groups of rats given 10, 100, or 500 ppm of ADN in their drinking water showed no effect on the indices of fertility, gestation, and viability through 3 generations.

Reference: Svirbely, J. L. (1963-1964). Am. Ind. Hyg. Conf. Abst.

Reliability: Not assignable because limited study information was available.

Additional Reference for Reproductive Toxicity:

Data from this additional source were not summarized because the study design was not adequate.

DuPont Co. (1950). Unpublished Data, Haskell Laboratory Report No. 20-50.

5.5 Genetic Toxicity

Type:	<i>In vitro</i> Bacterial Reverse Mutation Test
Tester Strains:	<i>Salmonella typhimurium</i> strains TA1535, TA1537, TA1538, TA98
Exogenous Metabolic Activation:	With and without rat liver S-9 homogenate
Exposure Concentrations:	0, 100, 500, 1000, 2500, 5000, 7500, 10,000 µg/plate
Method:	In the absence of metabolic activation, 0.1 mL of a solution of the test substance and approximately 10^8 bacteria were added to 2 mL of top agar. The solution was mixed and poured on the surface of an agar plate. The metabolic activation system involved the addition of 0.5 mL of S-9 mixture. The S-9 mixture contained S-9, MgCl ₂ , KCl, glucose 6-phosphate, NADP, and sodium phosphate. This mixture was added directly to the top agar immediately before it was poured over the minimal agar plate. Duplicate plates were used in each test condition.
	Prior to testing for mutagenicity, the test substance was tested for toxicity to the tester strains.
	Ethanol was used as the negative control. Positive control substances used in the test included 2-aminoanthracene, N-methyl-N'-nitro-N-nitrosoguanidine, 9-aminoacridine, and 2-nitrofluorene.
GLP:	No
Test Substance:	Adiponitrile, purity not specified
Results:	Negative
Remarks:	The test substance did not significantly increase the spontaneous, or background, mutation frequency.
Reference:	DuPont Co. (1976). Unpublished Data, Haskell Laboratory Report No. 87-76.
Reliability:	High because a scientifically defensible or guided method was used.

Additional References for *In vitro* Bacterial Reverse Mutation Studies:

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

NIOSH (1978). Criteria for a Recommended Standard Occupational Exposure to Nitriles, U.S. Dept. Health, Education and Welfare, Rockville, MD (IRIS/459).

Zeller, H. et al. (1969). Zentralbl. Arbeitsmed. Arbeitsschutz, 19(8):225-238.

Data from this additional source were not summarized because the test substance was a mixture or otherwise inappropriate.

Monsanto Co. (1980). TSCA Fiche OTS0515381 and OTS0515382.

Type:	<i>In vitro</i> Mouse Lymphoma Forward Mutation Test
Cell Type:	L5178Y mouse lymphoma cells, heterozygous for thymidine kinase (TK)
Exogenous Metabolic Activation:	With and without Aroclor-induced rat liver S-9
Exposure Concentrations:	0, 0.3, 0.5, 0.7, 0.85, 1, 2, 3, 5 µL/mL
Method:	Positive control substances used in the study included ethyl methanesulfonate (EMS) and 3-methylcholanthrene (3-MC). The vehicle control was diluent solvent, dimethyl sulfoxide (DMSO).

An initial test was conducted in the presence and absence of S-9 at 18 concentrations ranging from 1 to 5 µL/mL. The results of this test were used to determine the cytotoxicity of the test substance and the concentration range to use in the mutagenesis assays.

The experimental procedure for the mutagenesis assay was adapted from one described by Brown and Clive, 1978; Clive et al., 1972; 1973; 1979; Clive and Spector, 1975; Clive and Voytek, 1977. Triplicate samples were used for each test substance concentration and for the negative and positive controls. For each sample, 6×10^6 L5178Y TK^{+/−} cells in 10 mL of medium were prepared in a centrifuge tube. Each sample exposed to the test substance received 100 µL of the concentrated stock (100X). The centrifuge tubes were rotated for 4 hours in a roller drum at 37°C. After the exposure period, the treatment solutions were removed from the cells by a series of low-speed centrifugations, each followed by

removal of the supernatant and resuspension of the cells. Finally the cells were resuspended with the cell population density being to 4×10^5 , and 5% CO₂ in air was flushed through each tube. The centrifuge tubes were rotated in a roller drum for 2 days for expression of any mutations. After the expression period, 3×10^6 cells were seeded in cloning medium supplemented with trifluorothymidine (TFT) and 600 cells were seeded in nonselective cloning medium to determine viability. After cultivation of the cells for 11 days at 37°C in the presence of 5% CO₂ in air, the colonies of cells in each petri dish were counted with an automatic colony counter.

In order for the outcome of the test to be positive, the following criteria had to be met:

The mean mutation frequencies of at least 2 test samples containing concentrations (X_T), with a mean relative total growth value of at least 10%, were significantly greater than that of the solvent control.

The linear component of the dose-response relationship was statistically significant and exhibited a positive slope for test sample concentrations with a mean relative total growth of at least 10%.

For the test to be negative, both of the criteria below had to be met:

None of the mean mutation frequencies of any of the test sample concentrations were significantly greater than that of the solvent control.

The linear component of the dose-response relationship was not statistically significant for test sample concentrations with a mean relative total growth of at least 10%.

GLP:	Yes
Test Substance:	Adiponitrile, purity not specified
Results:	Negative
Remarks:	No tested concentration was cytotoxic. At the highest concentration tested (5 µL/mL), mean relative total growth values of 98 and 170% were obtained. No concentration, either with or without metabolic activation, displayed a mean mutation frequency significantly greater than that of the solvent controls, nor was a significant linear dose-response relationship observed.

- Reference: Monsanto Co. (1982). SRI International Project LSC-2575, April, TSCA Fiche OTS0516562.
- Brown, M. M. M. and D. Clive (1978). Mutat. Res., 53:116 (cited in Monsanto, 1982).
- Clive, D. et al. (1972). Mutat. Res., 16:77-87 (cited in Monsanto, 1982).
- Clive, D. et al. (1973). Chemical Mutagens: Principles and Methods for Their Detection, Vol. 1, pp. 79-103, Plenum Press, New York (cited in Monsanto, 1982).
- Clive, D. et al. (1979). Mutat. Res., 59:61-108 (cited in Monsanto, 1982).
- Clive, D. and J. F. S. Spector (1975). Mutat. Res., 31:17-29 (cited in Monsanto, 1982).
- Clive, D. and P. Voytek (1977). Mutat. Res., 44:269-278 (cited in Monsanto, 1982).
- Reliability: High because a scientifically defensible or guideline method was used.

Additional References for *In vitro* Studies:

Data from these additional sources were not summarized because the test substance was a mixture or otherwise inappropriate.

Monsanto Co. (1986). SRI International Project LSC-8747-5, July 10, TSCA Fiche OTS0515384.

Monsanto Co. (1986). Study No. PH 320-MO-004-85 (PK-850310), TSCA Fiche OTS0515387.

Type: ***In vivo* Bone Marrow Chromosome Aberration Study in Rats**

Species/Strain: Rats/Sprague-Dawley, CD[®]

Sex/Number: Male and female/15 per sex per dose group

Route of Administration: Gavage

Concentrations: 0, 300 mg/kg

Method: Rats were approximately 46-51 days old upon arrival, and acclimated for 12 days before exposures. Animals were housed individually and had food and water available *ad libitum*. The vehicle control group, consisting of 15 male

and 15 female rats, were administered corn oil. A positive control group, consisting of 5 male and 5 female rats, were administered 40 mg/kg of cyclophosphamide. The test substances were freshly prepared on the day of administration.

Observations of general appearance, behavior, toxic and pharmacologic effects, and body weights were recorded.

At approximately 4, 22, and 46 hours after administration of the test and control substances, designated rats (5 male and 5 female) received a single intraperitoneal injection of colchicine to inhibit mitosis and arrest cells in metaphase. Approximately 2 hours after injection of colchicine, the animals were sacrificed. The animals administered the positive control substance were sacrificed at the 24-hour sacrifice time only.

Immediately following sacrifice, bone marrow cells were prepared according to the modified techniques described by Evans, 1976 and Killian et al., 1977, fixed, and stained with Giemsa. An attempt was made to examine at least 50 cells in metaphase from each rat. In some instances, it was not possible to locate 50 spreads, so as many spreads as could be found were analyzed. The slides were scanned with a low power objective (10 or 25X) to find cells in the metaphase stage of mitosis and the chromosomes were analyzed with high power oil immersion lens (100X). The following items were recorded for each animal: numbers and types of chromosome aberrations, mitotic index, chromosome number for each metaphase and the vernier location of each metaphase containing damage.

GLP:	Yes
Test Substance:	Adiponitrile, purity 98%
Results:	Negative
Remarks:	ADN was not clastogenic in rat bone marrow cells; there were no statistically significant increases in the frequency of chromosomal aberrations compared to controls. No statistically significant differences were seen between the mean chromosome numbers and the mean mitotic indices for the test groups and the vehicle controls.

A statistically significant increase in percent aberrant cells, the average number of aberrations per cell, mean mitotic index, and in mean chromosome numbers was seen in the cyclophosphamide positive control groups.

Males administered 300 mg/kg showed severe signs of toxicity including depression, soft feces, rough coat, hunched posture, red stains on nose/eyes, labored respiration, and urine stains. Females administered 300 mg/kg showed much less severe signs of toxicity than did the males. These signs included slight depression, urine stains, and salivation. One male rat administered 300 mg/kg died while on study. Body weight losses were also observed in the 300 mg/kg males.

Reference: Monsanto Co. (1985). Hazelton Biotechnologies Corp. Project No. 84-221, TSCA Fiche OTS0514856.

Evans, H. J. (1976). Cytological Methods for Detecting Chemical Mutagens; Chemical Mutagenesis; Principles and Methods for Their Detection, Vol. 4, pp. 1-30, Plenum Press, New York (cited in Monsanto Co., 1985).

Killian, D. J. et al. (1977). A Collaborative Study to Measure Interlaboratory Variation with the *In vivo* Bone Marrow Metaphase Procedure, Handbook of Mutagen Testing, pp. 243-260, Elsevier, North-Holland, Amsterdam (cited in Monsanto Co., 1985).

Reliability: High because a scientifically defensible or guideline method was used.

Additional References for *In vivo* Studies:

Data from this additional source supports the study results summarized above. The study was not chosen for detailed summarization because the data were not substantially additive to the database.

Monsanto Co. (1985). SRI International Project LSC-7795, April, TSCA Fiche OTS0516565.

Data from this additional source were not summarized because the test substance was a mixture or otherwise inappropriate.

Pharmakon Research International, Inc. (1986). May 29, TSCA Fiche OTS0515383.

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APPENDIX B

ROBUST SUMMARY FOR 2-METHYLGLUTARONITRILE

The studies listed below were selected to represent the best available study design and execution for these HPV toxicity endpoints. Other data of equal or lesser quality are not summarized, but are listed as additional references in this document.

1.0 Substance Information

CAS Number: 4553-62-2

Chemical Name: Pentanedinitrile, 2-methyl

Structural Formula:

$$\text{N}\equiv\text{C}-\text{CH}_2-\text{CH}_2-\overset{\text{CH}_3}{\underset{|}{\text{CH}}}-\text{C}\equiv\text{N}$$

Other Names:

- 1,3-Dicyanobutane
- 2,4-Dicyanobutane
- 2-Methylglutaronitrile
- 2-Methyl-1,5-valerodinitrile
- alpha-Methylglutarodinitrile
- alpha-Methylglutaronitrile
- Diacrylonitrile
- Methyl glutaronitrile
- MGN
- 2-MGN

Exposure Limits: 1 ppm, (8-hour TWA), skin: DuPont Acceptable Exposure Limit (AEL)

2.0 Physical/Chemical Properties

2.1 Melting Point

Value:	-44 to -48°C (85% 2-MGN)
Decomposition:	No Data
Sublimation:	No Data
Pressure:	760 mm Hg
Method:	No Data
GLP:	Unknown
Reference:	DuPont Co. (1998). Material Safety Data Sheet No. DU005963 (September 18).
Reliability:	Not assignable because limited study information was available.

Additional Reference for Melting Point:

Butachimie (1993). Material Safety Data Sheet (October 14) (cited in IUCLID (1995). IUCLID Data Sheet “2-methylglutaronitrile” (October 23)).

2.2 Boiling Point

Value: 274°C (85% 2-MGN)
Decomposition: No Data
Pressure: 760 mm Hg
Method: No Data
GLP: Unknown
Reference: DuPont Co. (1998). Material Safety Data Sheet No. DU005963 (September 18).
Reliability: Not assignable because limited study information was available.

Additional References for Boiling Point:

Lewis, R. J., Sr. (ed.) (1996). Sax's Dangerous Properties of Industrial Materials, Van Nostrand Reinhold, New York.

Butachimie (1993). Material Safety Data Sheet (October 14) (cited in IUCLID (1995). IUCLID Data Sheet “2-methylglutaronitrile” (October 23)).

2.3 Density

Value: 0.95 g/mL (85% 2-MGN)
Temperature: 25°C
Method: No Data
GLP: Unknown
Results: No additional data.
Reference: DuPont Co. (1998). Material Safety Data Sheet No. DU005963 (September 18).
Reliability: Not assignable because limited study information was available.

Additional References for Density:

Butachimie (1993). Material Safety Data Sheet (October 14) (cited in IUCLID (1995). IUCLID Data Sheet “2-methylglutaronitrile” (October 23)).

Sigma Aldrich (1985). Library of Chemical Safety Data, 1st ed., Lenga RE (cited in IUCLID (1995). IUCLID Data Sheet “2-methylglutaronitrile” (October 23)).

2.4 Vapor Pressure

Value: 5.1×10^{-3} mm Hg (purity not specified)
Temperature: 25°C
Decomposition: No Data
Method: No Data
GLP: Unknown
Reference: Daubert, T. E. and R. P. Danner (1991). Physical and Thermodynamic Properties of Pure Chemicals Design Inst. Phys. Prop. Data, Amer. Inst. Chem. Eng., Vol. 2, Hemisphere Publishing Corp., New York (HSDB/6502).
Reliability: Not assignable because limited study information was available.

Additional References for Vapor Pressure:

DuPont Co. (1998). Material Safety Data Sheet No. DU005963 (September 18).

Butachimie (1993). Material Safety Data Sheet, October 14 (cited in IUCLID (1995). IUCLID Data Sheet "2-methylglutaronitrile" (October 23)).

2.5 Partition Coefficient (log Kow)

Value: -0.644 (purity not specified)
Temperature: No Data
Method: Estimated
GLP: No
Reference: U. S. EPA (1987). PCGEMS. PCLOGP Database, Office of Toxic Substances, Washington, DC (HSDB/6502).
Reliability: Estimated value based on accepted model.

Additional References for Partition Coefficient (log Kow): None Found.

2.6 Water Solubility

Value: 40 g/L (purity not specified)
Temperature: 20°C
pH/pKa: No Data
Method: No Data
GLP: Unknown
Reference: Butachimie (1993). Material Safety Data Sheet (October 14) (cited in IUCLID (1995). IUCLID Data Sheet "2-methylglutaronitrile" (October 23)).
Reliability: Not assignable because limited study information was available.

Additional Reference for Water Solubility:

DuPont Co. (1998). Material Safety Data Sheet No. DU005963 (September 18).

2.7 Flash Point

Value: 98°C (85% 2-MGN)
Method: Closed Cup
GLP: Unknown
Reference: DuPont Co. (1998). Material Safety Data Sheet No. DU005963 (September 18).
Reliability: Not assignable because limited study information was available.

Additional References for Flash Point:

Butachimie (1986). Material Safety Data Sheet (September) (cited in IUCLID (1995). IUCLID Data Sheet “2-methylglutaronitrile” (October 23)).

Sigma Aldrich (1985). Library of Chemical Safety Data, 1st ed., Lenga RE (cited in IUCLID (1995). IUCLID Data Sheet “2-methylglutaronitrile” (October 23)).

2.8 Flammability

Results: 0.3% – 3.3% (in air); Autoflammability = 538°C (purity not specified)
Method: No Data
GLP: Unknown
Reference: Butachimie (1986). Material Safety Data Sheet (September) (cited in IUCLID (1995). IUCLID Data Sheet “2-methylglutaronitrile” (October 23)).
Reliability: Not assignable because limited study information was available.

Additional Reference for Flammability:

DuPont Co. (1998). Unpublished Data, Material Safety Data Sheet No. DU005963 (September 18).

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3.0 Environmental Fate

3.1 Photodegradation

Concentration: Not Applicable
Temperature: Not Applicable
Direct Photolysis: Not Applicable
Indirect Photolysis: Not Applicable
Breakdown
Products: Not Applicable
Method: Based on a vapor pressure of 5.1×10^{-3} mm Hg (Daubert and Danner, 1991), 2-methylglutaronitrile should be found predominantly in the vapor phase in the atmosphere (Eisenreich et al., 1981; SRC, n.d.). In the atmosphere, vapor phase 2-methylglutaronitrile should react with photochemically produced hydroxyl radicals. Based on an estimation method (Atkinson, 1988), the rate constant for the reaction of 2-methylglutaronitrile with hydroxyl radicals has been estimated to be 1.208×10^{-12} cm³/molecule-sec (Atkinson, 1988). Assuming the daily average concentration of hydroxyl radicals in the atmosphere as 5×10^5 cm³ (Atkinson, 1985), the half-life for this reaction has been estimated to be 13.3 days. Based on a high water solubility estimated from log Kow (U. S. EPA, 1987) and a regression equation (Lyman, 1985), the removal of atmospheric 2-methylglutaronitrile by wet deposition should be important (SRC, n.d.).

GLP: Not Applicable
Reference: Daubert, T. E. and R. P. Danner (1991). Physical and Thermodynamic Properties of Pure Chemicals Design Inst. Phys. Prop. Data, Amer. Inst. Chem. Eng., Vol. 2, Hemisphere Publ. Corp., NY (HSDB/6502).

Eisenreich, S. J. et al. (1981). Environ. Sci. Technol., 15:30-38 (HSDB/6502).

SRC (Syracuse Research Corporation) (n.d.). (HSDB/6502).

Atkinson, R. (1988). Environ. Toxicol. Chem., 7:435-442 (HSDB/6502).

Atkinson, R. (1985). Chem. Rev., 85:69-201 (HSDB/6502).

U. S. EPA (1987). PCGEMS. PCLOGP Database, Office of Toxic Substances, Washington, DC (HSDB/6502).

Lyman, W. J. (1985). Environmental Exposure from Chemicals, Vol. 1, W. R. Neeley and G. E. Blau (eds.), p. 35, CRC Press, Boca Raton, FL (HSDB/6502).

Reliability: Estimated value based on accepted model.

Additional References for Photodegradation: None Found.

3.2 Stability in Water

Concentration: Not Applicable
Half-life: Not Applicable
% Hydrolyzed: Not Applicable
Method: Based on hydrolysis rates of a monosubstituted organic nitrile, hydrolysis of 2-methylglutaronitrile may not be important in environmental waters (Brown et al., 1975; SRC, n.d.). From the estimated Henry's Law constant (Hine and Mookerjee, 1975) and an estimation method (Lyman et al., 1982), the volatilization half-life of 2-methylglutaronitrile from a model river (1 m deep, flowing at 1 m/sec, and a wind speed of 3 m/sec) has been estimated to be 1259 days (Lyman et al., 1982; SRC, n.d.). Therefore, volatilization from water should not be important. The estimated log K_{oc} of 1.03 (Lyman et al., 1982) indicates that adsorption of 2-methylglutaronitrile to suspended solids and sediments in water should not be important (Swann, 1983).

GLP: Not Applicable
Reference: Brown, S. L. et al. (1975). Research Program on Hazard Priority Ranking of Manufactured Chemicals (Chemicals 41-60), p. 85, NTIS PB-263-163, SRI, Menlo Park, CA (HSDB/6502).

SRC (Syracuse Research Corporation) (n.d.). (HSDB/6502).

Hine, J. and P. K. Mookerjee (1975). J. Org. Chem., 40:292-298 (HSDB/6502).

Lyman, W. J. et al. (1982). Handbook of Chemical Property Estimation Methods, pp. 4-9, 5-5, and 15-21, McGraw-Hill, New York, NY (HSDB/6502).

Swann, R. L. (1983). Res. Rev., 85:17-28 (HSDB/6502).

Reliability: Estimated value based on accepted model.

Additional References for Stability in Water: None Found.

3.3 Transport (Fugacity):

Media:	Air, Water, Soil, Sediments
Distributions:	Air: 0.875%
	Water: 45.9%
	Soil: 53.2%
	Sediment: 0.0765%
Adsorption Coefficient:	Not Applicable
Desorption:	Not Applicable
Volatility:	Not Applicable
Method:	Calculated according to Mackay, Level III, Syracuse Research Corporation Epiwin Version 3.05. Emissions (1000 kg/hr) to air, water and soil compartments using EPA model defaults.
	Data Used:
	Molecular Weight: 108.14
	Henry's Law Constant: $2.97\text{E-}8 \text{ atm-m}^3/\text{mole}$ (Henry program)
	Vapor Pressure: $5.1\text{E-}3 \text{ mm Hg}$ (Daubert and Danner, 1991)
	Log Kow : -0.644 (U.S. EPA, 1987)
	Soil Koc : 0.781 (Log Kow estimate)
GLP:	No
Reference:	Daubert, T. E. and R. P. Danner (1991). <u>Physical and Thermodynamic Properties of Pure Chemicals Design Inst. Phys. Prop. Data</u> , Amer. Inst. Chem. Eng., Vol. 2, Hemisphere Publishing Corp., New York (HSDB/6502).
	U. S. EPA (1987). <u>PCGEMS. PCLOGP Database</u> , Office of Toxic Substances, Washington, DC (HSDB/6502).
	Syracuse Research Corporation EPIWIN v3.05 contains a Level III fugacity model. The methodology and programming approach was developed by Dr. Donald Mackay and co-workers which is detailed in:
	Mackay, D. (1991). <u>Multimedia Environmental Models: The Fugacity Approach</u> , pp 67-183, Lewis Publishers, CRC Press.
	Mackay, D. et al. (1996). <u>Environ. Toxicol. Chem.</u> , 15(9):1618-1626.
	Mackay, D. et al. (1996). <u>Environ. Toxicol. Chem.</u> , 15(9):1627-1637.
Reliability:	Estimated value based on accepted model.

Additional References for Transport (Fugacity): None Found.

3.4 Biodegradation: No Data.

3.5 Bioconcentration

Value: BCF = 0.2. The estimated BCF of 0.2 suggests that bioconcentration in aquatic organisms should be unimportant (SRC, n.d.).

Method: BCF was based on an estimated log Kow of -0.644 (U.S. EPA, 1987) and a regression equation (Lyman et al., 1982).

GLP: Not Applicable

Reference: U. S. EPA (1987). PCGEMS. PCLOGP Database, Office of Toxic Substances, Washington, DC (HSDB/6502).

Lyman, W. J. et al. (1982). Handbook of Chemical Property Estimation Methods, pp. 4-9, 5-5, and 15-21, McGraw-Hill, New York, NY (HSDB/6502).

Reliability: SRC (Syracuse Research Corporation) (n.d.). (HSDB/6502). Estimated value based on accepted model.

Additional References for Bioconcentration: None Found.

4.0 Ecotoxicity

4.1 Acute Toxicity to Fish:

Type: **96-hour LC₅₀**

Species: Freshwater fish

Value: 3317 mg/L

Method: Modeled, ECOSAR

GLP: Not Applicable

Test Substance: 2-methylglutaronitrile

Results: No Data

Reference: Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).

Reliability: Estimated value based on accepted model.

Additional References for Acute Toxicity to Fish: None Found.

4.2 Acute Toxicity to Invertebrates

Type: 24-hour EC₅₀
Species: *Daphnia magna* (water flea)
Value: 1550 mg/L
Method: No Data
GLP: Unknown
Test Substance: 2-methylglutaronitrile, purity not specified
Results: No additional data.
Reference: Rhone-Poulenc (1983). Unpublished Data (cited in IUCLID (1995). IUCLID Data Sheet "2-methylglutaronitrile" (October 23)).
Reliability: Not assignable because limited study information was available.

Additional References for Acute Toxicity to Invertebrates: None Found.

4.3 Acute Toxicity to Aquatic Plants:

Type: 96-hour EC₅₀
Species: Green algae
Value: 1787 mg/L
Method: Modeled, ECOSAR
GLP: Not Applicable
Test Substance: 2-methylglutaronitrile
Results: No Data
Reference: Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability: Estimated value based on accepted model.

Additional References for Acute Toxicity to Aquatic Plants: None Found.

5.0 Mammalian Toxicity

5.1 Acute Toxicity

Type: Oral LD₅₀
Species/Strain: Rats/Sprague Dawley
Value: 205 mg/kg (range of 174-243 mg/kg, Litchfield –Wilcoxon)
Method: Similar to OECD Guideline 401. Fasted rats were administered the test substance in 10% arabic gum.
GLP: Unknown

Test Substance: 2-methylglutaronitrile (technical), purity not specified
Results: All doses (100 – 400 mg/kg) induced hypoactivity, piloerection, and tremors up to 6 hours after administration. The LD₁₀₀ dose was 400 mg/kg. Death occurred within 2-6 hours.
Reference: Rhone-Poulenc (n.d.). Unpublished Data (Etude IFREB n_107 209) (cited in IUCLID (1995). IUCLID Data Sheet “2-methylglutaronitrile” (October 23)).
Reliability: Medium because a suboptimal study design was used and limited information was available.

Additional References for Acute Oral Toxicity:

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

DuPont Co. (1974). Unpublished Data, Haskell Laboratory Report No. 438-74.

DuPont Co. (1974). Unpublished Data, Haskell Laboratory Report No. 367-74.

Zeller, H. (1969). Zentralbl. Abreitsmed. Arbeitsschutz, 19(8):225-238 (CA71:128340).

DuPont Co. (1977). Unpublished Data, Haskell Laboratory Report No. 926-77.

Data from this additional source were not summarized because the test substance was a mixture or otherwise inappropriate.

DuPont Co. (1982). Unpublished Data, Haskell Laboratory Report No. 656-82.

Type: **Inhalation LC₅₀**
Species/Strain: Male rats/ChR-CD
Exposure Time: 4 hours
Value: 0.66 mg/L (149 ppm) (95% confidence limits, 0.39-1.34 mg/L)
Method: Six male rats were exposed to 0.33, 0.34, 0.49, or 1.11 mg/L for a single 4-hour period. All survivors were weighed and observed daily for 14 days post-exposure. The test substance was metered using an infusion pump into a stainless steel tube which was heated to 200°C. Houseline air, also metered through a heated line, was used to carry the test substance to the 20-L exposure chamber. The chamber atmospheres were analyzed quantitatively for 2-methylglutaronitrile. Known volumes were drawn through midjet impingers containing ethanol. These solutions were analyzed directly by gas chromatography. No histopathological examinations of tissues

were conducted. The LC₅₀ value was determined by the method of Finney.

GLP: No

Test Substance: 2-Methylglutaronitrile/ethyl succinonitrile (85%/14%)

Results: Mortality was observed in 2/6, 0/6, 3/6, and 5/6 rats exposed to 0.33, 0.34, 0.49, and 1.11 mg/L, respectively. Salivation, lacrimation, hyperemia, labored respiration, and mild convulsions were observed at non-lethal levels. At lethal levels, severe convulsions, exophthalmos, and cyanosis were followed by death immediately to 1 day post-exposure.

Reference: DuPont Co. (1974). Unpublished Data, Haskell Laboratory Report No. 574-74 (also cited in TSCA Fiche OTS0546553).

Reliability: High because a scientifically defensible or guideline method was used.

Additional References for Acute Inhalation Toxicity:

Data from this additional source were not summarized because insufficient study information was available.

Zeller, H. (1969). Zentralbl. Abreitsmed. Arbeitsschutz, 19(8):225-238 (CA71:128340).

Data from these additional sources were not summarized because the focus of the study was to determine if the substance was a Class B poison.

DuPont Co. (1984). Unpublished Data, Haskell Laboratory Report No. 232-84.

DuPont Co. (1978). Unpublished Data, Haskell Laboratory Report No. 27-78 (also cited in TSCA Fiche OTS0590011).

Type: **Dermal LD₅₀**

Species/Strain: Rabbits/New Zealand White

Exposure Time: 24 hours

Value: 776 mg/kg

Method: Five groups of 6 rabbits were clipped free of hair over the back and trunk area and fitted with plastic collars. Dose levels of 650, 725, 800, 1000, and 1500 mg/kg were tested. The test material was applied to intact skin on the back of each rabbit under a single gauze pad. The trunk of each rabbit was then wrapped with a layer of plastic wrap, stretch gauze bandage, and elastic adhesive tape. After a 24-hour period, the wrappings were removed, and the rabbits wiped with a dry towel. The rabbits were observed and/or weighed daily (except weekends) over a 14-day recovery period and then sacrificed.

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GLP:	No
Test Substance:	2-Methylglutaronitrile/ethyl succinonitrile (85%/14%)
Results:	Mortality occurred in 0/6, 5/6, 4/6, 3/6, and 6/6 rabbits at dose levels of 650, 725, 800, 1000, and 1500 mg/kg, respectively. Weight loss occurred at all dose levels tested. Prostration and gasping occurred in the 725 mg/kg dose group. All deaths occurred within 2 days after dosing.
Reference:	DuPont Co. (1983). Unpublished Data, Haskell Laboratory Report No. 39-83.
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Acute Dermal Toxicity:

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Rhone-Poulenc (n.d.). Unpublished Data (Etude IFREB n_107 209) (cited in IUCLID (1995). IUCLID Data Sheet "2-Methylglutaronitrile" (October 23)).

DuPont Co. (1977). Unpublished Data, Haskell Laboratory Report No. 847-77.

DuPont Co. (1984). Unpublished Data, Haskell Laboratory Report No. 537-84.

Type:	Dermal Irritation
Species/Strain:	Rabbit/Strain not specified
Method:	The test substance was placed either on the backs of rabbits for 15 minutes and washed, or left on their backs for 24 hours without washing.
GLP:	No
Test Substance:	2-methylglutaronitrile, purity not specified
Results:	No irritation was observed.
Reference:	Zeller, H. (1969). <u>Zentralbl. Arbeitsmed. Arbeitsschutz</u> , 19(8):225-238 (CA71:128340).
Reliability:	Not assignable because limited study information was available.

Additional References for Acute Dermal Irritation:

Data from this additional source support the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

Rhone-Poulenc (n.d.). Unpublished Data, Etude IFREB n_104 333 (cited in IUCLID (1995). IUCLID Data Sheet "2-methylglutaronitrile" (October 23)).

Data from these additional sources were not summarized because the focus of the study was skin corrosion.

DuPont Co. (1974). Unpublished Data, Haskell Laboratory Report No. 194-74.

DuPont Co. (1974). Unpublished Data, Haskell Laboratory Report No. 298-74.

DuPont Co. (1977). Unpublished Data, Haskell Laboratory Report No. 945-77.

Type: **Dermal Sensitization:** No Data.

Type: **Eye Irritation**

Species/Strain: Male rabbits/Albino

Method: 0.1 mL of the undiluted test material was placed in the right conjunctival sac of each of 2 male albino rabbits. After 20 seconds, one treated eye was washed with tap water for 1 minute. The treated eye of the other rabbit was not washed. Observations of the cornea, iris, and conjunctiva were made with a hand-slit lamp at 1 and 4 hours, and at 1, 2, and 3 days.

GLP: No

Test Substance: 2-Methylglutaronitrile/ethyl succinonitrile (85%/14%)

Results: A small area of minimal opacity and mild conjunctival irritation with no irritic effect in the unwashed eye occurred. The corneal opacity was reversible and the eye was normal within 3 days. An eye dosed with the compound and promptly washed had a small area of minimal opacity and very mild conjunctival irritation with no irritic effect and was normal within 2 days.

Reference: DuPont Co. (1974). Unpublished Data, Haskell Laboratory Report No. 411-74.

Reliability: High because a scientifically defensible or guideline method was used.

Additional References for Acute Eye Irritation:

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Zeller, H. (1969). Zentralbl. Arbeitsmed. Arbeitsschutz, 19(8):225-238 (CA71:128340).

Rhone-Poulenc (n.d.). Unpublished Data, Etude IFREB n_104 333 (cited in IUCLID (1995). IUCLID Data Sheet "2-methylglutaronitrile" (October 23)).

5.2 **Repeated Dose Toxicity:** 4-week inhalation study in progress.

5.3 **Developmental Toxicity:** No Data.

5.4 **Reproductive Toxicity:** No Data.

5.5 **Genetic Toxicity**

Type: *In vitro* Bacterial Reverse Mutation Assay

Tester Strains: *Salmonella typhimurium* TA97, TA98, TA100, TA1535, and TA1537

Exogenous
Metabolic

Activation: With and without Aroclor[®]-induced rat and hamster liver S-9
Exposure

Concentrations: 0, 100, 333, 1000, 3333, 10,000 µg/plate

Method: The preincubation assay was performed as described in Haworth, S. et al. (1983). Environ. Mutagen., 5(Suppl. 1):3-142, with some minor modifications. The test substance, *Salmonella* culture, and S-9 mix or buffer were incubated at 37°C, without shaking, for 20 minutes. The top agar was added and the contents of the tubes were mixed and poured onto the surface of petri dishes containing medium. The histidine-independent (his+) colonies arising on these plates were machine counted following 2 days incubation at 37°C, unless precipitate was present that interfered with the count, or the color of the test substance on the plate reduced the contrast between the colonies and the background agar. Plates with low numbers of colonies were counted by hand.

Concurrent solvent and positive controls were run with each trial.

A test substance was judged mutagenic or weakly mutagenic if it produced a reproducible dose-related response over the solvent control in replicate trials. A test substance was judged questionable if the results of individual trials were not reproducible, if increases in his+ revertants did not meet criteria for a weakly mutagenic response, or if only single doses produced increases in his+ revertants in repeat trials. A test substance was judged nonmutagenic if it did not meet the criteria for a mutagenic or questionable response.

GLP: Unknown

Test Substance: 2-methylglutaronitrile, purity 85%

Results: Weakly mutagenic

Remarks: No additional data.

Reference: Zeiger, E. et al. (1988). Environ. Mol. Mutagen., 11(Suppl. 12):1-158.
Reliability: High because a scientifically defensible or guideline method was used.

Additional Reference for *In vitro* Bacterial Reverse Mutation Assays:

Data from this additional source support the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

Rhone-Poulenc (n.d.). Unpublished Data, Etude CERTI n_541 (cited in IUCLID (1995). IUCLID Data Sheet "2-methylglutaronitrile" (October 23)).

Type: *In vitro* Clastogenicity Assay: No Data.

Type: *In vivo* Mouse Micronucleus Test

Species/Strain: Mice/Strain not specified

Sex/Number: Male/10 per group

Route of

Administration: Oral gavage

Concentrations: 47.5 and 95 mg/kg

Method: Mice were treated twice and sacrificed 6 hours after the last gavage. Bone-marrow slides were prepared from the femur to analyze 2000 polychromatic erythrocytes (PCEs) per animal for the presence of micronuclei (MN).

GLP: Unknown

Test Substance: 2-methylglutaronitrile, purity not specified

Results: Negative

Remarks: At 95 mg/kg, animals died within 2 hours. Some death occurred with the first administration at 47.5 mg/kg. No increase in micronucleated PCEs was noted in the exposed groups when compared with the control group. The positive control, methyl methane sulfonate (MMS), showed a significant increase in the frequency of MN.

Reference: Rhone-Poulenc (n.d.). Unpublished Data, Etude CERTI n_541 (cited in IUCLID (1995). IUCLID Data Sheet "2-methylglutaronitrile" (October 23)).

Reliability: Medium because a suboptimal study design was used and limited study information was available.

Additional References for *In vivo* Studies: None Found.

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APPENDIX C

ROBUST SUMMARY FOR 2-ETHYLSUCCINONITRILE

The studies listed below were selected to represent the best available study design and execution for these HPV toxicity endpoints. Other data of equal or lesser quality are not summarized, but are listed as additional references in this document.

1.0 Substance Information

CAS Number: 17611-82-4

Chemical Name: Butanedinitrile, ethyl

Structural Formula:

$$\text{N}\equiv\text{C}-\text{CH}_2-\overset{\text{C}_2\text{H}_5}{\underset{|}{\text{CH}}}-\text{C}\equiv\text{N}$$

Other Names: 2-Ethylsuccinonitrile
Ethylsuccinonitrile

Exposure Limits: No Data

2.0 Physical/Chemical Properties

2.1 Melting Point:

Value:	-39 to -43°C
Decomposition:	No
Sublimation:	No
Pressure:	No Data
Method:	Laboratory method for freezing point
GLP:	Yes
Reference:	DuPont Co. (1986). Unpublished Data.
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Melting Point: None Found.

2.2 Boiling Point:

Value:	264°C
Decomposition:	No
Pressure:	1 atm
Method:	Antoine Constants by Buillometric technique
GLP:	Yes
Reference:	DuPont Co. (1985). Unpublished Data.
Reliability:	High because a scientifically defensible or guideline method

was used.

Additional References for Boiling Point: None Found.

2.3 Density:

Value: 0.948 g/mL
Temperature: 25°C
Method: Pycnometer
GLP: Yes
Results: No additional data.
Reference: DuPont Co. (1986). Unpublished Data.
Reliability: High because a scientifically defensible or guideline method was used.

Additional References for Density: None Found.

2.4 Vapor Pressure:

Value: 0.019 mm Hg
Temperature: 25°C
Decomposition: No
Method: Antoine Constants by Buillometric technique. Antoine Constants A=17.82333, B=5506.134, C=227.756.
GLP: Yes
Reference: DuPont Co. (1985). Unpublished Data.
Reliability: High because a scientifically defensible or guideline method was used.

Additional Reference for Vapor Pressure:

SRC MPBPWin v1.40 Syracuse Research Corporation (MPBPWIN) program.

2.5 Partition Coefficient (log Kow):

Value: 0.28
Temperature: No Data
Method: Estimated via the Kowwin program
GLP: Unknown
Reference: The Log Octanol-Water Partition Coefficient Program (KOWWIN) estimates the logarithmic octanol-water partition coefficient (log P) of organic compounds. The KOWWIN program and estimation methodology were developed at Syracuse Research Corporation. KOWWIN uses a "fragment constant" methodology to predict log P. A journal article by Meylan and Howard (1995) describes the program

methodology (Meylan, W.M. and P.H. Howard (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients).

Reliability: Estimated value based on acceptable model.

Additional References for Partition Coefficient (log Kow): None Found.

2.6 Water Solubility:

Value: 2.2 wt% (GC analysis) (22 g/L)
Temperature: 23°C
pH/pKa: Neutral
Method: pH meter
GLP: Yes
Reference: DuPont Co. (1986). Unpublished Data.
Reliability: High because a scientifically defensible or guideline method was used.

Additional References for Water Solubility: None Found.

2.7 Flash Point: No Data.

2.8 Flammability: No Data.

3.0 Environmental Fate

3.1 Photodegradation:

Concentration: Not Applicable
Temperature: Not Applicable
Direct: Not Applicable
Indirect: Not Applicable
Breakdown: Not Applicable
Products:
Method: Based on a estimated vapor pressure of 0.019 mm Hg, 2-ethylsuccinonitrile should be found predominately in the vapor phase of the atmosphere (SRC MPBPWin v1.40). In the atmosphere, vapor phase 2-ethylsuccinonitrile should react with photochemically produced hydroxyl radicals. The rate constant for the reaction of 2-ethylsuccinonitrile with hydroxyl radicals has been estimated to be 1.58×10^{-12} cm³/molecule-sec. Assuming the daily average concentration of hydroxyl radicals in the atmosphere as 1.5×10^6 OH/cm³, the half-life for this reaction has been estimated to be 6.7 days (12-hr day).
GLP: Not Applicable
Reference: Syracuse Research Corporation EpiWin v3.05 contains an Atmospheric Oxidation Program (AOP) which estimates the

rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. The estimation methods used by the program are based upon the structure-activity relationship (SAR) methods developed by Dr. Roger Atkinson and co-workers. The AOP program is described in:

Meylan, W.M. and Howard, P.H. (1993). Chemosphere, 26:2293-99.

Reliability: Estimated value based on accepted model.

Additional References for Photodegradation: None Found.

3.2 Stability in Water:

Concentration: Not Applicable
 Half-life: Not Applicable
 % Hydrolyzed: Not Applicable
 Method: Based on an estimated Henry's Law constant (Bond SAR method SRC EpiWin), the volatilization half-life of 2-ethylsuccinonitrile from a model river (1 m deep, flowing at 1 m/s, and a wind velocity of 5 m/s) has been estimated to be 854.2 days. The half-life of 2-ethylsuccinonitrile from a model lake (1 m deep, with a current velocity of 0.05 m/s and wind velocity of 0.5 m/s) has been estimated to be 9322 days. Therefore, volatilization from water should not be an important fate process.
 GLP: Not Applicable
 Reference: Syracuse Research Corporation EpiWin v3.05 contains a Volatilization Rate from Water Model (WVOL). The WVOL program estimates the volatilization half-lives from a model river and lake using the methodology from the Handbook of Chemical Property Estimation Methods (Lyman, W. J., Reehl, W. F., and Rosenblatt, D. H., American Chemical Society, 1990) Estimation Handbook (adsorption to suspended solids and sediments is ignored).
 Reliability: Estimated value based on accepted model.

Additional References for Stability in Water: None Found.

3.3 Transport (Fugacity):

Media: Air, Water, Soil, Sediments
 Distributions: Air: 0.861%
 Water: 45.3 %
 Soil: 53.8%

	Sediment:	0.767%
Adsorption Coefficient:	Not Applicable	
Desorption:	Not Applicable	
Volatility:	Not Applicable	
Method:	Calculated according to Mackay, Level III, Syracuse Research Corporation Epiwin Version 3.05. Emissions (1000 kg/hr) to air, water, and soil compartments using standard EPA model defaults.	
	Data Used:	
	Molecular Weight:	108.14
	Henry's Law Constant:	2.97×10^{-8} atm-m ³ /mole (HenryWin)
	Vapor Pressure:	0.019 mm Hg (DuPont, 1985)
	Log Kow :	0.28 (Kowwin program)
	Soil Koc :	0.781 (Log Kow estimate)
GLP:	Not Applicable	
Reference:	DuPont Co. (1985). Unpublished Data.	
	Syracuse Research Corporation EPIWIN v3.05 contains a Level III fugacity model. The methodology and programming approach was developed by Dr. Donald Mackay and co-workers which is detailed in:	
	Mackay, D. (1991). <u>Multimedia Environmental Models; The Fugacity Approach</u> , pp. 67-183, Lewis Publishers, CRC Press.	
	Mackay, D. et al. (1996). <u>Environ. Toxicol. Chem.</u> , 15(9):1618-1626.	
	Mackay, D. et al. (1996). <u>Environ. Toxicol. Chem.</u> , 15(9):1627-1637.	
Reliability:	Estimated value based on accepted model.	

Additional References for Transport (Fugacity): None Found.

3.4 Biodegradation: No Data.

3.5 Bioconcentration:

Value:	BCF = 3.162 (log BCF 0.5). This estimated BCF suggests the potential for bioconcentration in aquatic organisms is low.
Method:	Bioconcentration factor (BCF) was calculated by BCFWIN Computer Program, Version 2.13, Syracuse Research Corporation. The estimated value was calculated using a log Kow of 0.28 and a regression-derived equation.

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GLP: Not Applicable
Reference: The estimation methodology used by BCFWIN is described in the following document prepared for the U.S. Environmental Protection Agency (OPPT):

"Improved Method for Estimating Bioconcentration Factor (BCF) from Octanol-Water Partition Coefficient", SRC TR-97-006 (2nd Update), July 22, 1997; prepared for: Robert S. Boethling, EPA-OPPT, Washington, DC; Contract No. 68-D5-0012; prepared by: William M. Meylan, Philip H. Howard, Dallas Aronson, Heather Printup and Sybil Gouchie; Syracuse Research Corp., Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212.
Reliability: Estimated value based on accepted model.

Additional References for Bioconcentration: None Found.

4.0 Ecotoxicity

4.1 Acute Toxicity to Fish:

Type: **96-hour LC₅₀**
Species: Freshwater fish
Value: 3317 mg/L
Method: Modeled, ECOSAR
GLP: Not Applicable
Test Substance: Ethylsuccinonitrile
Results: No additional data.
Reference: Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability: Estimated value based on accepted model.

Additional References for Acute Toxicity to Fish: None Found.

4.2 Acute Toxicity to Invertebrates:

Type: 48-hour EC₅₀
Species: *Daphnia magna*
Value: 831 mg/L (95% fiducial limits, 338 to 2678 mg/L)
Method: The acute toxicity to *Daphnia magna* (less than 24 hours old) was determined in an unaerated, 48-hour, static test. The study was conducted with 4 concentrations (1.0, 10, 100, and

1000 mg/L) and a dilution water control at a mean temperature of 20.4°C. One test chamber was used per test concentration with 10 test organisms in each chamber.

GLP: No

Test Substance: Ethylsuccinonitrile, approximately 98%

Results: All water quality parameters were within acceptable limits during the exposure. Based on visual observations, the water control and the test concentrations were clear and colorless at test start. Exposure to the test substance resulted in 0, 0, 0, 0, and 60% immobility for the 0, 1.0, 10, 100, and 1000 mg/L groups, respectively, at 48 hours.

Reference: DuPont Co. (2000). Unpublished Data, Haskell Laboratory Report No. DuPont-5196.

Reliability: Medium because a suboptimal study design was used that did not include measurement of test substance concentrations during the study.

Additional References for Acute Toxicity to Invertebrates: None Found.

4.3 Acute Toxicity to Aquatic Plants:

Type: **96-hour EC₅₀**

Species: Green algae

Value: 1787 mg/L

Method: Modeled, ECOSAR

GLP: Not Applicable

Test Substance: Ethylsuccinonitrile

Results: No additional data.

Reference: Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).

Reliability: Estimated value based on accepted model.

Additional References for Acute Toxicity to Aquatic Plants: None Found.

5.0 Mammalian Toxicity

5.1 Acute Toxicity:

Type: **Oral Fixed Dose**

Species/Strain: Male rats/ChR-CD

Value: The estimated minimum lethal dose was > 50 mg/kg and ≤ 500

mg/kg.

Method: In a preliminary study, a single dose of ESN, mixed with deionized water, was administered to fasted female rats (1/group) at doses of 50 or 500 mg/kg. Mortality, body weight effects, and clinical signs of toxicity were observed for 8 days after dosing. The rats were approximately 9 weeks old on the day of dosing.

In the main study, single doses of ESN, mixed with deionized water, were administered by intragastric intubation to a group of 5 fasted males and a group of 5 fasted female rats at a dose of 50 mg/kg. The rats were observed for mortality, body weight effects, and clinical signs for 14 days after dosing. The rats were necropsied to detect grossly observable evidence of organ or tissue damage or dysfunction. The male rats were approximately 7 weeks old and the female rats were approximately 12 weeks old on the day of dosing.

GLP: Yes

Test Substance: Ethylsuccinonitrile, purity approximately 98%

Results: In the preliminary study, the rat dosed with 500 mg/kg died. No adverse clinical signs of toxicity or significant body weight loss occurred in the female rat dosed with 50 mg/kg.

In the main study, no deaths occurred during the study. The rats exhibited no clinical signs of toxicity or significant body weight loss during the study. No gross lesions were observed in the rats at necropsy.

Reference: DuPont Co. (2001). Unpublished Data, Haskell Laboratory Report No. DuPont-5525.

Reliability: High because a scientifically defensible or guideline method was used.

Additional References for Acute Oral Toxicity:

Data from these additional sources were not chosen for detailed summarization because the test substance was a mixture or otherwise inappropriate.

DuPont Co. (1974). Unpublished Data, Haskell Laboratory Report No. 438-74.

DuPont Co. (1977). Unpublished Data, Haskell Laboratory Report No. 926-77.

Type: **Inhalation ALC**

Species/Strain: Male rats/Crl:CD[®](SD)IGS BR

Exposure Time: 4 hours

Value: 1.4 mg/L

Method: Chamber atmospheres were generated by aerosolization of the

test substance in air with a Spraying Systems nebulizer. The test substance was metered into the nebulizer with a pump. Filtered, houseline air, introduced at the nebulizer, atomized the test substance and carried the aerosol into the exposure chamber. Chamber concentrations were controlled by varying the test substance feed rate to the atmosphere generator. Atmospheric concentrations were determined by gravimetric analysis at approximately 30-minute intervals. Samples to determine particle size distribution were taken. Airflow, oxygen concentration, temperature, and relative humidity in the chamber were monitored and recorded.

Groups of 6 male rats were exposed to the atmospheres in a 19 L glass chamber for single 4-hour exposures at concentrations of 0.91, 1.4, or 2.5 mg/L. Rats were weighed and observed for clinical signs of toxicity during a 14- or 15-day recovery period. Rats were approximately 8-9 weeks old and weighed between 234 and 298 grams at the time of exposure.

GLP:

No

Test Substance:

Ethyl succinonitrile, purity approximately 98%

Results:

Rats were exposed to atmospheres at concentrations of 0.91, 1.4, or 2.5 mg/L. The mass median aerodynamic diameters for the aerosol tested ranged from 2.6 to 3.6 μm . Chamber temperature ranged from 22 to 25°C, chamber relative humidity ranged from 25 to 31%, chamber airflow was 18 L/min, and the oxygen concentration ranged from 21 to 22%. Although chamber temperature and relative humidity were outside the targeted parameters, these deviations were not considered to have adversely affected the results of the study.

Rats died following exposure to ethylsuccinonitrile at concentrations of 1.4 mg/L and greater. Deaths occurred during exposure or within 6 days of exposure.

Notable clinical signs of toxicity observed during the study included hunched posture, red nasal and ocular discharges, and clear, red, or brown discharges around the mouth. These signs were typically observed by test day 1 and had resolved by test day 3. No clinical signs of toxicity were observed in the 0.91 mg/L group.

Weight losses were observed in all groups following exposure. Rats generally exhibited slight to severe (3.4-15%) body weight losses within 1 day of exposure but began to regain

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weight by test day 3.
Reference: DuPont Co. (2001). Unpublished Data, Haskell Laboratory Report No. DuPont-5152.
Reliability: High because a scientifically defensible or guideline method was used.

Additional References for Acute Inhalation Toxicity:

Data from these additional sources were not chosen for detailed summarization because the test substance was a mixture or otherwise inappropriate.

DuPont Co. (1974). Unpublished Data, Haskell Laboratory Report No. 574-74 (also cited in TSCA Fiche OTS0546553).

DuPont Co. (1984). Unpublished Data, Haskell Laboratory Report No. 232-84.

DuPont Co. (1978). Unpublished Data, Haskell Laboratory Report No. 27-78 (also cited in TSCA Fiche OTS0590011).

Type: **Acute Dermal Toxicity:** No Data.

Additional Reference for Acute Dermal Toxicity:

Data from these additional sources were not chosen for detailed summarization because the test substance was a mixture or otherwise inappropriate.

DuPont Co. (1983). Unpublished Data, Haskell Laboratory Report No. 39-83.

DuPont Co. (1977). Unpublished Data, Haskell Laboratory Report No. 847-77.

Type: **Dermal Irritation:** No Data.

Additional References for Acute Dermal Irritation:

Data from these additional sources were not summarized because the test substance was a mixture or otherwise inappropriate.

DuPont Co. (1974). Unpublished Data, Haskell Laboratory Report No. 194-74.

DuPont Co. (1974). Unpublished Data, Haskell Laboratory Report No. 298-74.

DuPont Co. (1977). Unpublished Data, Haskell Laboratory Report No. 945-77.

Type: **Dermal Sensitization:** No Data.

Type: **Eye Irritation:** No Data.

Additional Reference for Acute Eye Irritation:

Data from this additional source were not summarized because the test substance was a mixture or otherwise inappropriate.

DuPont Co. (1974). Unpublished Data, Haskell Laboratory Report No. 411-74.

5.2 Repeated Dose Toxicity: No Data.

5.3 Developmental Toxicity: No Data.

5.4 Reproductive Toxicity: No Data.

5.5 Genetic Toxicity:

Type: *In vitro* Bacterial Reverse Mutation Test

Tester Strains: *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *E. coli* strain WP2 *uvrA*

Exogenous

Metabolic

Activation: With and without Aroclor[®]-induced rat liver S-9 homogenate

Exposure

Concentrations: 0, 100, 333, 1000, 3333, 5000 µg/plate

Method: A preliminary toxicity test was conducted to establish the dose-range over which the test substance would be assayed. Vehicle and 6.7, 10, 33, 67, 100, 333, 667, 1000, 3333, and 5000 µg/plate of the test substance were used.

The mutagenicity test initial assay (Experiment B1), repeat assay (Experiment B2), and independent repeat assay (Experiment B3) were used to evaluate the mutagenic potential of the test substance. All dose levels of the test substance, vehicle controls, and positive controls were plated in triplicate. Positive control substances tested in this study included 2-aminoanthracene, 2-nitrofluorene, sodium azide, 9-aminoacridine, and methyl methanesulfonate. The reaction mixture (S-9 mix) contained glucose 6-phosphate, NADP, KCL, MgCl₂, and S-9 in a phosphate buffer.

The test system was exposed to the test substance via the plate incorporation methodology described by Ames et al., 1975 and updated by Maron and Ames, 1983. Treatments with activation were conducted by adding 0.5 mL of S-9 mix, 100 µL of tester strain and 50 µL of vehicle, positive control, or test substance to 2 mL of top agar at 45±2°C. After

vortexing, the mixture was overlaid onto the surface of 25 mL of minimal bottom agar. After the overlay had solidified, the plates were inverted and incubated for approximately 48 to 72 hours at $37\pm 2^{\circ}\text{C}$. Treatments in the absence of the metabolic activation system were identical to those with activation with the exception that a Sham mix (100 mM phosphate buffer at pH 7.4) was used as a replacement for the S-9.

Bacterial background lawns were evaluated for evidence of test substance toxicity and precipitation. Revertant colonies for a given tester strain and condition, except for positive controls, were counted either entirely by an automated colony counter or entirely by hand unless the test was the preliminary toxicity test or the plate exhibited toxicity. Plates with sufficient test substance precipitate to interfere with automated colony counting were counted manually.

Dimethyl sulfoxide was selected as the solvent of choice based on solubility of the test substance and compatibility with the target cells.

For the test substance to be classified as positive, it must have caused a dose-related increase in the mean revertants per plate of at least one tester strain with a minimum of two increasing concentrations of test substance. Data sets for strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 3 times the mean vehicle control value. Data sets for strains TA98, TA100, and WP2 *uvrA* were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 2 times the mean vehicle control value.

GLP:	Yes
Test Substance:	Ethylsuccinonitrile, purity approximately 98%
Results:	Negative
Remarks:	In the preliminary toxicity test, no precipitate or toxicity was observed at the maximum dose tested, 5000 μg per plate.
	In the mutagenicity test, no precipitate or toxicity was observed. No positive responses were observed with any of the tester strains in the presence or absence of S-9 activation in either the initial mutagenicity assay or in the independent repeat assay.
Reference:	DuPont Co. (2001). Unpublished Data, Haskell Laboratory Report No. DuPont-5101.

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Ames, B. N. et al. (1975). Mutat. Res., 31:347-364.

Maron, D. M. and B. N. Ames (1983). Mutat. Res., 113:173-215.

Reliability: High because a scientifically defensible or guideline method was used.

Type: ***In vitro* Chromosome Aberration Assay:** No Data.

Type: ***In vivo* Chromosome Aberration Assay:** No Data.